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SYMPOSIUM on BIOLOGICAL EFFECTS and MEASUREMENT of RADIO FREQUENCY/MICROWAVES

Proceedings of a Conference
held in Rockville, Maryland
February 16-18, 1977

Edited by
DeWitt G. Hazzard, Ph.D.

BRH
bureau of radiological health

WHO Collaborating Center for
Standardization of Protection
Against Nonionizing Radiations

July 1977

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Food and Drug Administration
Bureau of Radiological Health
Rockville, Maryland 20852

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FOREWORD

The Bureau of Radiological Health conducts a national program to limit man's exposure to ionizing and nonionizing radiations. To this end, the Bureau (1) develops criteria and recommends standards for safe limits of radiation exposure, (2) develops methods and techniques for controlling radiation exposure, (3) plans and conducts research to determine health effects of radiation exposure, (4) provides technical assistance to agencies responsible for radiological health control programs, and (5) conducts an electronic product radiation control program to protect the public health and safety.

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> WHO Collaborating Center for Standardization of Protection Against Nonionizing Radiations (Office of the Bureau Director)
>
> WHO Collaborating Center for Training and General Tasks in Radiation Medicine (Division of Training and Medical Applications)
>
> WHO Collaborating Center for Nuclear Medicine (Division of Radioactive Materials and Nuclear Medicine)

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Bureau publications provide an effective mechanism for disseminating results of intramural and contractor projects. The publications are distributed to State and local radiological health personnel, Bureau technical staff, Bureau advisory committee members, information services, industry, hospitals, laboratories, schools, the press, and other concerned individuals. These publications are for sale by the Government Printing Office and/or the National Technical Information Service.

Readers are encouraged to report errors or omissions to the Bureau. Your comments or requests for further information are also solicited.

John C. Villforth
Director
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EDITOR'S NOTE

The papers included in these Proceedings of the Symposium of Biological Effects and Measurement of Radio Frequency/Microwaves were submitted by the authors as written versions of papers presented at the Symposium. The open discussions are from verbatim transcription made during the Symposium. Transcripts of both the papers and open discussions that followed their presentations were returned to the authors for editing and verification of accuracy. Where there was a substantive deviation from the original transcription, the editor exercised his prerogative in declining some of the proposed changes to the open discussion that were submitted by the authors.

The editor gratefully acknowledges the cooperation of the many who made this effort a success, including those who accepted invitations, not only to speak at this Symposium but to provide manuscripts as well for these Proceedings. They certainly stimulated some very interesting and informative discussions among themselves and other participants, to whom I am grateful.

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PREFACE

The Bureau of Radiological Health conducts scientific and technical evaluation of radiation-emitting electronic products as one segment of the Food and Drug Administration's implementation of the Radiation Control for Health and Safety Act of 1968, P.L. 90-602. Bureau efforts also include development and evaluation of a wide variety of methods of analysis for appraising the health impact of radiation exposure. This Symposium is but another means of meeting this objective.

The goals of this Symposium were many-fold. In the standard tradition, it provided a forum for scientific exchange. However, this Symposium had its unique aspects, as well. By holding the meeting at BRH headquarters, opportunity was provided for the extramurally supported investigators to meet firsthand with the total Bureau professional staff and exchange information, as well as become familiar with our research facilities. Engineers and biological scientists met and collectively discussed their problems and how they interface with one another. And finally, the scope of the BRH research effort in the microwave area, both intramural and extramural, is summarized in these Proceedings.

This Symposium has been the second one of a series planned to cover the various areas of BRH responsibility. On behalf of the Bureau, I would like to express thanks to Dr. DeWitt Hazzard, not only for his initiative in planning this meeting, but for the execution of a very successful session; and the cooperation of the many others who made this effort a success. The dedicated and highly professional effort of this group resulted in an outstanding meeting which will, I believe, be recognized as a significant milestone in the BRH tradition--as a means of program assessment, bringing together intramural and extramural staff in a research area, and understanding that particular topic area.

Robert H Neill

Robert H. Neill
Associate Director for Administration
Bureau of Radiological Health

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OPENING SESSION

WELCOME
DeWitt G. Hazzard

THE BRH PERSPECTIVE
Robert H. Neill

WELCOME

DeWitt G. Hazzard
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I am very delighted to see the very good turnout we have for this symposium--at least I think I am delighted. You know, it is sort of a mixed blessing. It is always very nice to see so many people interested; but then, when you get so many people interested, it creates problems with regard to parking and facility management. So, I believe I am happy to see you all here. Unless we are seeing a false positive reaction, I guess I would have to conclude from the large response that the area of biological effects and measurement of microwaves is one of great interest at the present time.

This symposium has essentially three objectives. One objective is to offer a platform or a forum for scientific interchange--the classic reason for holding such a meeting. However, beyond that we have a couple other objectives for having this symposium, particularly for having it at our headquarters. One purpose is to bring in our extramural research component and to give our research staff and our administrative staff an opportunity to meet with you people and to talk with you; so often grantees and contractors are just names out in the woods somewhere never to be seen.

The third objective is to publish the proceedings of the symposium, which will serve a couple purposes in itself. First, the proceedings will serve as the usual scientific reference that people can go to; and secondly, it will allow a documentation of exactly what our present research efforts are in the area of RF/microwaves. So often we receive inquiries from the "hill" as to what the Bureau is doing in this or what the Bureau is doing in that. Now, with such a record of proceedings one can very easily show that this is what we are doing.

The papers to be presented at this symposium over the next 3 days represent just about the totality of our extramural program and a very large portion of our intramural program within our RF/microwave research program. Some of these, I might add, are cofunded by the Navy. Speaking for myself, I must say that I am very proud of this program. I think as you look over the agenda, you will see a lot of people listed who have very fine reputations for the production of quality research; and I think it is a great honor that these are people that we are supporting.

With no further ado, I would like to introduce my boss, who is the Associate Bureau Director for Administration for the Bureau of Radiological Health: Mr. Robert Neill.

THE BRH PERSPECTIVE

Robert H. Neill
Associate Director for Administration
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I certainly want to add my comments to DeWitt's in welcoming you to Washington. We are very pleased that you have been able to take time from your very busy schedules to report on the progress of your work.

There certainly has been considerable interest expressed in this field. We are pleased by your presence today--heightened in part by Mr. Paul Brodeur's extraordinary in-depth articles in the microwave area. To reiterate DeWitt, the purpose of our symposium today is not only to provide a forum for scientific exchange and to discuss the Bureau's research, but to encourage the exchange of information between our own professional staff and those in the academic sector; and, of course, to provide a public accountability through the proceedings.

It is interesting to note that the mission or goal of the Bureau of Radiological Health has remained unchanged, essentially, since our creation in 1957; namely, to reduce or to eliminate all unnecessary radiation exposure to the American public. Certainly this symposium is intended to aid in fulfilling that mission. I might note that this is the second symposium to provide an accounting of FDA-sponsored research in the nonionizing radiation area, with the first symposium having been held in March 1976, entitled the Biological Effects in Measurement of Light Sources. We plan to hold a similar symposium on ultrasound later this year.

At this time I would like to provide an accounting of some of the dollar levels of support for the work being described at this symposium as a relevant perspective to you, both as scientists as well as taxpayers. Efforts supported by the Food and Drug Administration approximate $1.4 million, which is about 9 percent of the total Bureau resources. As I am sure you know, we have other areas of responsibility which have included limiting exposure from x-ray machines, lasers, ultrasound devices, x rays from color TV sets; and an educational program program geared to reduce unnecessary radiation exposure from diagnostic x-ray applications. One could view the level of funding in the microwave area as less than 1 cent per United States citizen. It might be interesting to note that the entire FDA budget of $225 million costs one dollar per person in the U.S. to insure safe and efficacious foods, drugs, cosmetics, and medical devices. It is always intriguing to me, as a public health official, that within FDA where we have been allotted one dollar per year for the prevention of ill effects in our population, one can compare this allotment to the average of $630 a year per person for the correction of ill health in our medical care programs. It does tend to show clearly that investments in the prevention of ill health is a best buy for the American public. One can also note that the increase of 15 percent a year in health care costs would triple the costs to $7,500 a year for a family of four in 9 years. I cite that as a perspective of the importance of preventing ill health.

The $1.4 million that I mentioned in microwave radiation may be broken down as follows: 1/3 in the form of contracts and grants and 2/3 for inhouse research. The inhouse studies amounting to $900,000 can further be subdivided into three pieces: compliance 35 percent, measurement 34 percent, and biological studies 31 percent. The compliance activities of our Division of

Compliance will not be described at this symposium, but they are the key element in insuring the protection of the public by adherence to the performance standard for microwave ovens. Their activities relate to report review, factory inspections, laboratory measurements, and quality control review. It is rather interesting to note that the direct compliance cost of $320,000 is only 20 percent of the total level of effort in FDA of $1.4 million, which may be surprising to you in an Agency reputed to be solely regulatory. However, the biological studies clearly are supportive to and are part of our compliance activity. I am speaking, initially, of just the hard core Division of Compliance efforts. It would be most economical if we could set a standard; get the enforcement program into good shape; and then go on to the next one. Unfortunately, this is not true; and our cumulative responsibilities in radiological health continue to increase. The studies being reported here certainly will provide feedback to our October 1971 microwave oven performance standard.

I might comment on the legislative basis for these efforts. Our interest in supporting the research stems from explicit responsibilities defined by Congress in the Radiation Control for Health and Safety Act of 1968, Public Law 90-602, to conduct and support research to minimize the emissions of and the exposure of people to unnecessary electronic product radiation; to study and evaluate emission and conditions of exposure to electronic product radiation; and to prescribe limitations on the emissions of electronic product radiation from such products if it is determined that such standards are necessary to protect the public health and safety. Since the scope of many of the scientific studies to be reported here are indirectly applicable to identifying the need of setting a performance standard, we have scheduled a panel for Friday afternoon, right after the symposium, to obtain your suggestions and advice regarding the identification of products and devices to be considered for regulation. The members of the panel are: Dr. Sol Michaelson, University of Rochester; Dr. Don Justesen, Kansas City Veterans Administration Hospital; Dr. Suzanne Bawin, University of California at Los Angeles; and Dr. Arthur Guy, University of Washington.

As you know there is a formal system in developing a standard to insure that all affected parties have the opportunity to present their views on the consequences of such a standard. This system provides an opportunity for consumers, users, manufacturers, and others, to comment. The proposal must be aired by public announcements in the FEDERAL REGISTER and considered by the 15 member Technical Electronic Product Radiation Safety Standards Committee, which is composed of five representatives from government, industry, and the public. Our symposium today is not intended to bypass that system; but we feel that since you are all here, it would be a missed opportunity not to take advantage of any advice that you may wish to provide at this time.

It might be interesting to compare some analogies to the ionizing radiation in the microwave field. While the mechanism of interaction with tissue are different and cannot directly be compared, there is an interesting series of points I would like to make.

1. It is estimated that the total expenditure in studying the biological effects of radiation is in excess of probably $1.5 billion or $2 billion since World War II. The annual level of funding in FDA, in assessing the consequences of RF/microwave radiation, as I mentioned before, is certainly on the order of $1.5 million.

2. While ionizing radiation sources are located in controlled environments, laboratories, hospitals, and universities, RF/microwave radiation sources are to be found not only in the controlled environment but in the

uncontrolled environment of the home as well, such as the microwave oven in the kitchen.

3. We have some of the problems associated with the difficulty of assessing behavioral effects in the nonionizing microwave radiation.

4. The proliferation of RF microwave devices has been a steep exponential which leads us to conclude that the population at risk tends to be almost the entire U.S. population.

5. With ionizing radiation we have always presumed that if the manmade exposures are equal to 1 or 2 times the natural background, basically we would be okay. Natural background from RF microwave is a negligible quantity. I might mention also that in the area of ionizing radiation, general population criteria have historically included a factor of safety of 300--below the level observed to have had an effect. Certainly we wish to insure that there is no degradation of protection via nonionizing radiation sources.

For these reasons it appears quite clear that the challenge to insure the protection of the public health and safety from microwaves and RF sources is a vitally important one. We hope that this symposium will help to provide a meaningful basis to insure that we act clearly and promptly in the discharge of those responsibilities.

SESSION II. BEHAVIORAL EFFECTS

INTRODUCTORY REMARKS
Moris L. Shore

BEHAVIORAL EFFECTS OF ELECTROMAGNETIC ENERGY
Allan H. Frey

FREE-OPERANT AVOIDANCE AND ESCAPE FROM MICROWAVE RADIATION
John C. Monahan and W. W. Henton

MICROWAVE ABSORPTION AND TASTE AVERSION AS A FUNCTION OF 915 MHz RADIATION
John C. Monahan and W. W. Henton

BEHAVIORAL PROTOCOLS AND PRELIMINARY OBSERVATIONS OF CHICKS EXPOSED TO WEAK AMPLITUDE MODULATED 450 MHz FIELDS
R. G. Medici and P. M. Sagan

MICROWAVE HYPERTHERMIA AND THE VISUALLY EVOKED POTENTIALS: PRELIMINARY OBSERVATIONS OF GUINEA PIGS
Don R. Justesen and Virginia Bruce-Wolfe

INTERACTION BETWEEN MICROWAVE AND NEUROACTIVE COMPOUNDS
W. D. Galloway and Morris Waxler

INTRODUCTORY REMARKS

Moris L. Shore
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I want to add my own words of welcome--I am taking the chairman's perogative for this first session--and also perhaps say a few words to you prior to our paper presentations.

In 1969, at the time that we held the Richmond symposium in conjunction with the development of our own regulatory activity with respect to microwaves, we saw a reawakening of the interest in microwaves which had lain dormant pretty much since the termination of the Tri-Service Program. The Tri-Service Program, in fact, had been initiated in 1957 and had reached its conclusion in the early 1960's (the significant portion of that program). It left us a legacy, primarily of thermal effects, and a very good appreciation for cataract as a significant endpoint of microwave exposure in animals. It left us also with a rationalization of 10 milliwatts per square centimeter as a standard for human exposure with what was considered to be a factor of safety based on the animal experimentation.

In a short period of 8 years to the present time, as knowledge has increased, we have seen this presumed factor of safety eroded on the basis of acute studies. Significant questions are still to be answered--questions that relate to the existence of cumulative effects--genetic effects, delayed effects. We have a need for human epidemiological studies--well designed studies that provide adequate followup of exposed and controlled populations. We have a crucial need for studies that examine low-level effects of chronic or lifetime exposure, particularly in animals, since this could not be done in man.

During a discussion that we had yesterday, a statement was made by one of the scientists present at this meeting: "We have just developed, at the present time, the technological competence to perform meaningful research in this area." Of course, one of the problems that we have had all along has been the question of dosimetry so that we can quantitate dose response. Scientists have been very preoccupied with questions that perhaps should be peripheral to this program--the questions of thermal versus specific or non-thermal effects. I think what we need to do is to scientifically and critically examine dose effect and dose response under conditions that are relevant to the human problem. We need to develop the scientific information that will make it possible for us to make public health and regulatory decisions on a rational basis.

There are mistakes that have been made in the past, and I would hope that these could be avoided. A specific example is the certification of safety of 10 milliwatts per square centimeter for indefinite human exposure in the absence of any studies in animals or in man that tested the chronic or lifetime effects of exposure to 10 milliwatts per square centimeter. Such certification, based on ignorance, strains the credibility of a level that is recommended as being adequate to protect public health and safety, particularly of the general population. We need to examine and to study with scientific objectivity the research that is done by scientists working in the field, regardless of where they are--whether it is in this country or abroad--Eastern Europe, Western Europe, or South America. We need to avoid the spectacle of blind acceptance of all negative studies and concerted criticism of studies showing positive effects at low levels. I think that all studies need to be examined critically, whether positive or negative, with complete scientific objectivity. Additionally, we need to avoid the spectacle of explaining everything away on the basis of, "well, this is thermal," and if we cannot explain it on the basis of "it is thermal, it is microthermal." Is this really what is relevant? Is this really what is important? I think these are questions that we need to ask ourselves. As a public health agency, we have a concern for public health and safety. It is only this concern that mandates our intramural and extramural scientific program in this area.

BEHAVIORAL EFFECTS OF ELECTROMAGNETIC ENERGY

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ABSTRACT

The results of two lines of investigation are presented. In one, the effect of exposure to electromagnetic energy on irritability and aggression in mammals is detailed. It was found that EM energy exposure elicits a "docility" response in a pain induced aggression situation. In the other series of experiments, it was found that exposure to EM energy adversely affects motor coordination or balance in the task of balancing on a rotating rod.

INTRODUCTION

Two lines of investigation are reported herein. One concerns irritability and aggression in mammals as affected by electromagnetic energy. The other is concerned with electromagnetic energy exposure effects on motor coordination or balance.

On the basis of our previous findings and reports from Eastern Europe, it was hypothesized that electromagnetic energy might affect irritability or aggressive behavior in mammals. Thus, a series of experiments was undertaken to explore the possibility that pain elicited aggressive behavior in rats would be affected by illumination with ultra high frequency electromagnetic energy.

EXPERIMENT I

Materials and Methods

Male Sprague-Dawley rats weighing approximately 250 grams were used. The rats were housed individually and maintained ad lib. They were randomly divided into two equal groups so that there could be ten bouts of fighting among the animals in each group to establish dominance. One group fought while exposed to EM energy and the other group fought during sham exposure.

The apparatus was a 20 cm sq x 30 cm high chamber constructed of 3 mm clear acrylic, as shown in figure 1. The chamber consisted of two sections. The lower section was 8.5 cm high and slightly flared toward the top. A 4 mm acrylic flange was located on the inside of and 3.5 cm below the top of the lower section. A 25 cm high clear acrylic upper section rested on this flange. Two 1.3 cm diameter holes were centered on opposite sides of the lower section, 3.5 cm above the chamber floor. When the animals were placed in the chamber, their tails were passed through these holes and held by Velcro tape to an acrylic bar mounted at right angle to the chamber side. This acrylic bar was mounted above the hole and was 6 cm long and 1.5 cm x 1.5 cm in thickness.

In this experiment and the ones that follow, ultrasonic output in the 23 to 28 kHz range was also recorded from the paired animals. An ultrasound sensor was placed at the top of the tail pinch chamber and it activated a pen on the

Esterline Angus event recorder. The chamber rested on two wooden blocks 9 cm high, which in turn rested on a wooden platform. The chamber was attached to the blocks by nylon bolts and nuts. The blocks were glued to the platform.

A lever intermittently compressed the tail against the bar. The lever was a wooden dowel 8.5 cm long and 1.3 cm in diameter. It was attached to and parallel to the side of the chamber below the tail hole. The lever was attached to the chamber side by a nylon bolt that passed through a hole in the lever 1 cm from the end, such that the lever could pivot on the bolt. The pivot bolt was located to the side of the tail hole 1.4 cm above the chamber floor and 5 cm from the nearest side. There was a similar assembly at each tail hole.

The tail compression levers were driven by a motor turning a 1.7 cm diameter dowel rod. The motor torque was 300 inch-ounces and speed was 20 RPM. The dowel rod passed under the chamber and turned in a bicycle bearing ring, mounted 7 cm above the platform surface. Two wooden discs were mounted eccentrically on and were glued to the dowel rod. The wooden discs were 4.5 cm in diameter and 1.5 cm thick. The rod passed through these discs 1.25 cm off center. The discs were so mounted as to lift and release the levers in unison as the motor drove the rod. During the experiments, the apparatus was on a wooden table in an rf anechoic enclosure. Tail pinch elicited aggressive behavior in the rats was scored by an observer who did not know if the exposure was real or sham. The observer recorded the occurrence and duration of each behavior by depressing a push button associated with a particular response category. The push buttons activated various pens on a multi-channel Esterline Angus event recorder. A method for the categorization and quantification of pain elicited aggressive behavior had been previously developed and is described below. Briefly, the repertoire of pain induced aggressive behaviors were divided into four mutually exclusive categories: pre-fight behavior, active fighting behavior A, active fighting behavior B, and dominant-submissive behavior. In Experiment I, categories A-AF and B-AF had not yet been separated. Descriptions of the behaviors associated with each category are as follows:

Pre-fighting behavior (PF). PF is characterized by stand-up boxing behavior by the paired rats. It is often preceded by audible squealing, teeth chattering, and hair ruffing (by which the rats can sometimes appear 20 percent larger). The animals raise their forepaws off the ground and face each other with their eyes open, whiskers typically touching and paws occasionally brushing. Their heads are back and raised upwards. The animals rock back and towards each other in unison, each occasionally leaning against the wall or losing its balance, but generally maintaining a non-deferential position to the other rat.

Active Fighting A (A-AF). Although this behavior may occur without PF behavior, its first occurrence usually follows a period of PF behavior. At a given point in the pre-fighting behavior, the subjects rock back and lunge forward. There follows a rolling, violent period of wrestling, scratching and biting. There is no obvious aggressor in this behavior category and it is usually impossible to identify a fight initiating rat. There is no squealing accompanying this behavior. A-AF behavior occasionally results in bloodshed, but typically this is limited to the paws which are engorged with blood. This behavior is usually brief (0.5 to 2 seconds in duration).

Active Fighting B (B-AF). This behavior is specific to pain elicited aggression. In fighting behavior in rats, a dominant-submissive relationship between the subjects rapidly appears. Typically, this dominant-submissive relationship ends fighting. In pain elicited aggression, however, the dominant animal may continue to attack the submissively postured animal. These attacks center on biting the submissive animal about the muzzle, throat and eyes, and are usually quite bloody. Sometimes an animal is killed. The attacks appeared to be directed against the struggles of the submissively postured animal to right itself. There are ultrasonic emissions associated with this behavior in the 23 to 28 kHz frequency band.

Dominant-Submissive Behavior. This category is limited to the postures and behaviors associated with the outcome of prior active fighting. Typically, one animal is poised over the other, standing on its hind legs and resting its paws on the submissive animal's chest. The submissive animal's eyes are usually closed and its throat is bared. The dominant animal will often groom the submissive one. Sometimes the dominant animal will merely lean over the other or even rest next to the submissive one while the latter remains in the typical submissive posture. There is a high correlation between this response category and ultrasound emission in the 23 to 28 kHz frequency band.

As mentioned above, there were ten combinations of bouts in each group. Two bouts per group were run per day and each bout was 5 minutes long. After all bouts were run, the sequence was repeated.

To begin a bout, a pair of animals were placed in the lower section of the tail pinch chamber. Their tails were drawn through the tail holes and fastened onto the lever stops with Velcro tape. The chamber top with the ultrasound transducer attached was lowered into place. The motor started, thus driving the levers against the rats' tails. The timing of the bout was begun when the motor was started. In the present experiment, one group was illuminated with EM energy while the other group was sham illuminated.

The energy was generated by a Microdot Model 411A oscillator and conveyed to a standard gain horn antenna. The carrier frequency was 1300 MHz with a pulse width of 0.5 ms and repetition rate of 1000 pps. The average power was 0.65 mW/cm^2 with a peak power of 1.3 mW/cm^2.

For each category of aggressive behavior, the illuminated and sham groups were compared on onset time, duration time, offset time, and total episodes.

Results

In general, the EM energy illuminated group was more "docile" than the sham illuminated group. The EM energy exposed group took significantly longer to begin aggressive behavior ($p < .05$). Duration of fighting of the exposed group was less than in the sham illuminated group ($p < .001$). The aggressive behavior of the exposed group ended sooner ($p < .01$). Episodes of fighting were significantly less for the exposed group ($p < .001$) as was frequency of submissive dominance behavior ($p < .05$). The results are shown in histogram form in figure 2.

EXPERIMENT II

Materials and Methods

The subjects were Sprague-Dawley rats weighing approximately 225 grams. They were housed separately and maintained ad lib. The rats were randomly divided into two equal groups. All bouts were within groups and 15 combinations of bouts were held for each group. The series was then repeated.

The apparatus and scoring criteria in Experiment II were similar to those used in Experiment I.

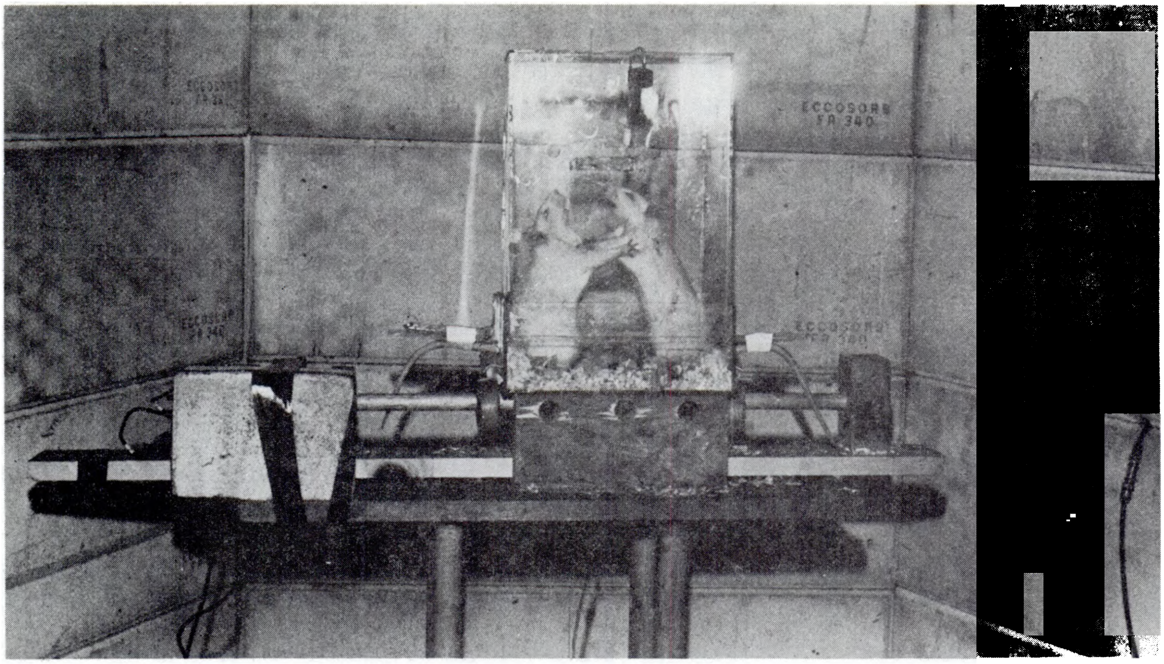
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Figure 1

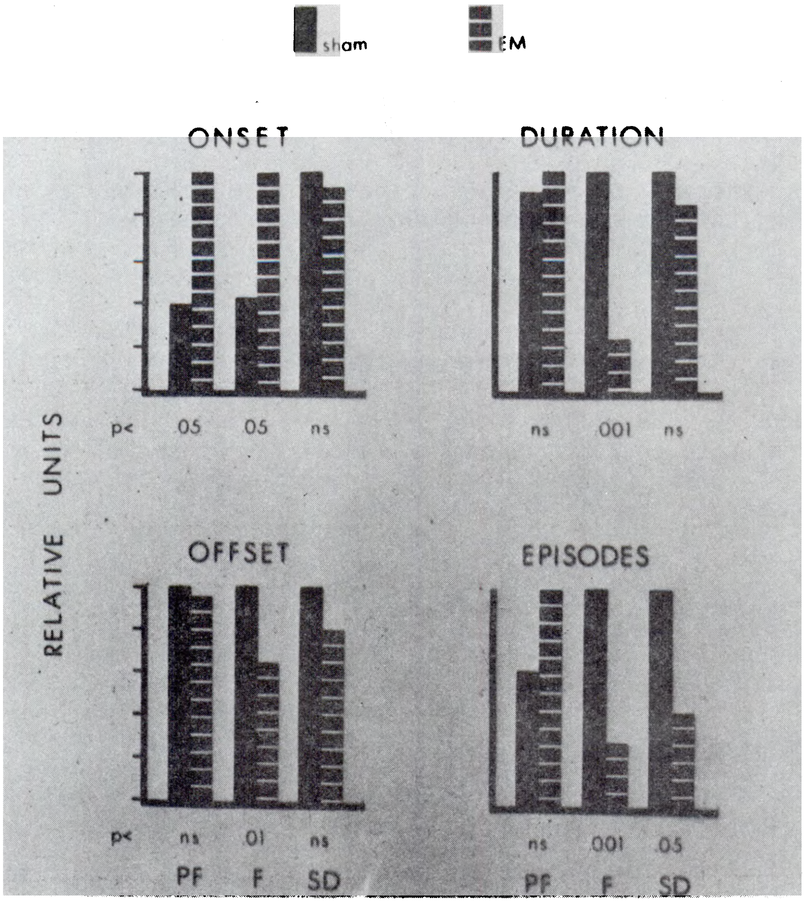

Figure 2

The procedure in Experiment II was also similar to that of Experiment I, except that the EM energy group was exposed to an average power density of 0.2 mW/cm^2 and a peak power density of 0.4 mW/cm^2.

Results

In figure 3, the combined data for series 1 and 2 is shown. They indicate that the EM energy exposed animals exhibit less aggression than do the sham exposed animals, i.e., shorter, less frequent aggressive episodes and a smaller mean amount of time spent in the aggressive behaviors. The exposed animals began and terminated the respective aggressive behaviors later than did the control animals. Considering the series 1 and 2 data separately, there was less fighting and pre-fighting behaviors in the second series of bouts compared to the first series, but the differences between sham illuminated and illuminated animals were similar.

EXPERIMENT III

Materials and Methods

The subjects were male Sprague-Dawley rats weighing approximately 225 grams. The animals were randomly divided into ten groups and were maintained ad lib. They were housed in pairs with animals of different groups sharing cages. Each rat fought only rats in its own group and there were six bouts per group. When the series of bouts was completed, the series was repeated.

The apparatus and behavioral categorization criteria were similar to that of Experiment II.

The procedure was similar to that of Experiment II except that the EM energy parameters were those noted in Table 1.

Table 1. Electromagnetic energy parameters used in Experiment III. In each case except the sham and CW exposures, the pulse repetition rate was 1000 pps.

Frequency GHz	Average power mW/cm^2	Peak power mW/cm^2	Pulse width ms
1.0	0.1	0.2	0.5
1.0	0.05	0.1	0.5
1.0	0.025	0.05	0.5
1.0	0.02	0.2	0.1
1.0	0.01	0.1	0.1
1.0	0.005	0.05	0.1
1.3	0.1	0.2	0.5
1.3	0.2	0.2	CW
1.5	0.1	0.2	0.5
Sham	0	0	--

Results

Experiment III was designed to explore which parameters of the EM energy are of consequence in the elicitation of the docility found in the previous experiments. The histograms in figure 4 indicate the effect of peak power on pain induced aggression. As was seen in Experiments I and II, EM energy illuminated animals show shorter, less frequent aggressive episodes when compared to sham exposed animals. EM energy illuminated animals start and terminate aggression later than sham exposed animals. The duration of aggressive behaviors is also less in EM energy exposed animals. There was less fighting and more submissive dominant behavior during the second series of bouts than occurred in the first series of bouts in Experiment III. The exposure effects are similar in both series.

The data suggest a positive linear relationship between the degree of docility and peak power employed. There are indications, however, that this may have been an inverted U-shaped function if the power had been increased into the milliwatt level.

There are indications that CW energy is not as effective as pulsed energy in eliciting docility, although both pulsed and CW energy exposed groups are significantly more docile than their sham exposed counterparts. The exact relationship was not defined in this experiment.

Discussion

A clear cut "docility" effect of exposure to EM energy has been found in animals undergoing the stress of the pain induced aggression procedure. The exact nature of the effect and the appropriate label for the effect remains to be defined. The effect occurs at rather low power levels as such are defined in biological investigations of EM energy effects. The power levels used, however, are above what is generally found in the environment. The threshold, for the EM energy parameters used, is a peak power between 0.05 mW/cm^2 and 0.1 mW/cm^2. The measurements given, although exact, are considered to be correct within an order of magnitude because of the perturbing effect of the animal and any apparatus in the EM field.

We note that the effect can be related in terms of carrier frequency, modulation, and power level to the effects of EM energy on the blood brain barrier reported by Frey, Feld and Frey (1). There is no justification in the present data, however, to assume a causal relationship between the two effects.

The other line of investigation concerned electromagnetic energy exposure effects on motor coordination or balance.

A substantial number of studies have shown that exposure to microwave frequency electromagnetic energy affects behavior (2, 3, 4). Several reports in this literature suggest that motor control or balance would be affected by pulse modulated electromagnetic energy. Frey, for example, has shown an auditory effect of EM energy which implies the possibility of labyrinthine organ effect (5). Lebovitz reports labyrinthine organ effects of the energy (6). Thus, a systematic series of experiments were undertaken to determine if motor coordination or balance is affected by the energy and the thresholds at certain energy parameters for such effects were assessed.

Materials and Methods

A horizontal rod, set to rotate at an increasing rate of speed until a predetermined rate of speed was reached, was designed for rats to balance on in

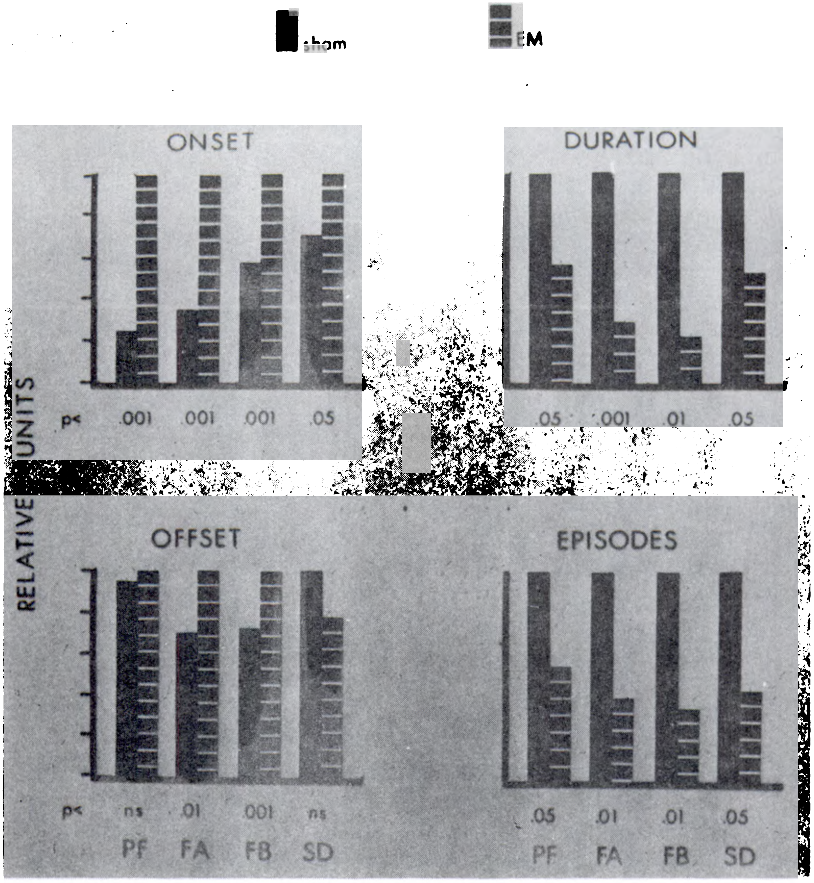

Figure 3

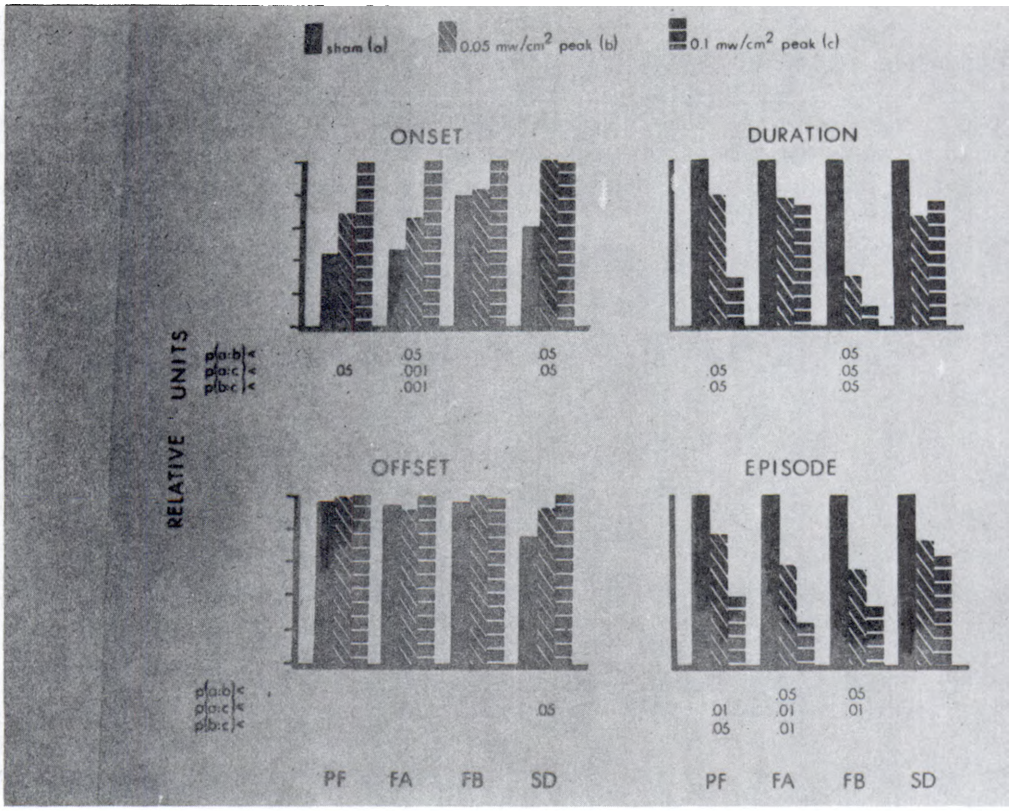

Figure 4

the EM energy field. The apparatus, shown in figure 5, consists of a rod, motor, and U frame. The rod was a 3.2 cm wooden dowel 40 cm long. A wooden U frame was constructed to support the rod which closed the top of the U. The frame consisted of a base measuring 56 cm square with two 112 cm high 2" x 4"'s as arms of the U. A motor was mounted near the top of one arm. The motor assembly used for the experiment was an RMS Motor Corporation variable speed gearmotor. A small fan was used for cooling the motor and as a pink noise source during the experiments. The motor and fan were mounted on a 25 cm x 12 cm x 4 cm block of wood which was attached to an arm of the U. The motor was oriented so that the shaft pointed to the opposite arm of the U. The shaft of the motor was set into one end of the horizontal wooden rod and secured with a pin. The other end of the rod was set into a bicycle wheel bearing mounted in a 5 cm x 10 cm block of wood. The wooden block was attached to the wooden arm opposite the motor-fan assembly. The metal components of the apparatus were shielded from the energy by use of anechoic material.

Two 30 cm x 23 cm x 0.65 cm plates of white styrene were suspended vertically from above the rod and divided the space on the rod into three sections. The three plates were notched at the bottom to fit over the rotating rod. They were suspended above the rod by the use of two 1 cm dowel rods passing through holes near the top of the plates and attached to the top of the arms of the U frame. These plates prevented an animal, placed between them, from walking off the end of the rod. A clear sheet of vinyl was suspended hammock style on a smaller wooden frame and placed under the rotating rod to catch the subjects as they fell from the rod.

The variable speed gearmotor was connected to a RMS Motor Corporation CB-1 speed control. The speed control was stepped with a stepping switch driven by an AEL Model 104A pulse generator. The pulse generator provided a pulse every nine seconds to the speed control thus increasing the rotation of the rod. The starting speed was 14 rpm with a maximum speed of 42 rpm being reached after the system was in operation for 90 seconds. At the end of two minutes or when the animal fell off, the stepper was turned off and re-set.

The energy was provided by a Microdot Model 411A pulse generator connected via RG 8 cable to a standard gain horn antenna. The frequencies used in this experimentation were 1 GHz, 1.3 GHz, and 1.5 GHz. The pulse repetition rate was 1000 pps, with a pulse width of 0.5 ms. The average power density measured in Experiment I was 1.4 mW/cm^2 with a peak power of 2.8 mW/cm^2. In Experiment II, the average power measured was 0.2 mW/cm^2 with a peak power of 0.4 mW/cm^2. Energy densities were measured before and after each session. The measurement was done with a dipole antenna set up above and just behind the rod. The dipole antenna was connected via RG-58 cable to a Microlab AF-20 attenuator located outside the chamber. The attenuator was connected to a Hewlett Packard Model 477B thermistor mount which was connected to a Hewlett Packard Model 430C power meter.

The testing was done in an rf anechoic chamber constructed of Eccosorb FR #340 with the exposure to EM energy occuring only during the time the experimental treatment animals were being tested on the rotating rod. A time record was kept from the start of the rotation until the animal lost its balance on the rod. Loss of balance was defined as falling from the rod or loss of footing on the rod, e.g. animal wrapped its body around the rod. The time on the rod was recorded in seconds with a time limit of 120 seconds. All conditions of testing for the illuminated and sham illuminated animals were the same except for the presence of the EM energy emitted from the Microdot 411A oscillator. All equipment was tested and adjusted for each session.

EXPERIMENT I

Procedure

The animals were tested over a multi-week period in six sessions. They were tested five times per session in 120 second runs. There was an experimental and control group for each frequency with six animals in each group.

Results

The mean time on the rod is shown in figure 6 for each frequency. The significance of the differences was tested with the use of the Mann-Whitney U test. There were significant differences between experimental and control group means at 1.3 GHz and 1.5 GHz. The difference between groups at the 1.0 GHz frequency was not significant. The means of the variances were compared by use of the Mann-Whitney U test. In figure 7, it may be noted that there is significantly less variance in the electromagnetic energy exposed groups at the 1.3 GHz and 1.5 GHz frequencies. The difference for the 1.0 GHz exposure was not significant.

At the end of the experiment, the rotation of the rod was reversed. The animals were placed on the rod as before, so that they were facing into the rotation. They were not, however, facing the horn as before. The EM energy exposed animals' behavior was quite disturbed and their performance became very erratic. It required five sessions before their performance began to stabilize. In contrast, the animals that were sham exposed readily adapted to the change and within the second session were performing as previously.

EXPERIMENT II

Procedure

In this experiment, new groups of animals were used. They were used in an own-control design with half illuminated with the energy first and half sham illuminated first. Exposure conditions were then reversed, after a week of no experimentation to allow possible residual effects to dissipate. This design was used because pilot testing had suggested that the power densities used in Experiment I were close to threshold and an effect on variance had been shown. In addition, we were reducing the power density to which the animals were exposed in Experiment II almost an order of magnitude below the exposure used in Experiment I. This design was the most sensitive design for determining effects at threshold level without the use of large groups.

In this factorial design, the animals were separated into two groups that were matched according to their ability on the wheel during pre-test trials. Group A was exposed to EM energy for the first half of the experiment and Group B was sham illuminated. For the second half of the experiment, the exposures were reversed. Using random selection, animals in Group A were assigned to three different EM carrier frequencies. The animals in Group B were assigned to the same three frequencies. The frequencies used were 1 GHz, 1.3 GHz, and 1.5 GHz. Testing consisted of twelve sessions with a one-week break at the half-way point. Each animal was given four trials on the rotating rad during each session. The sessions were held approximately every other day with the pause of one week between session 6 and 7.

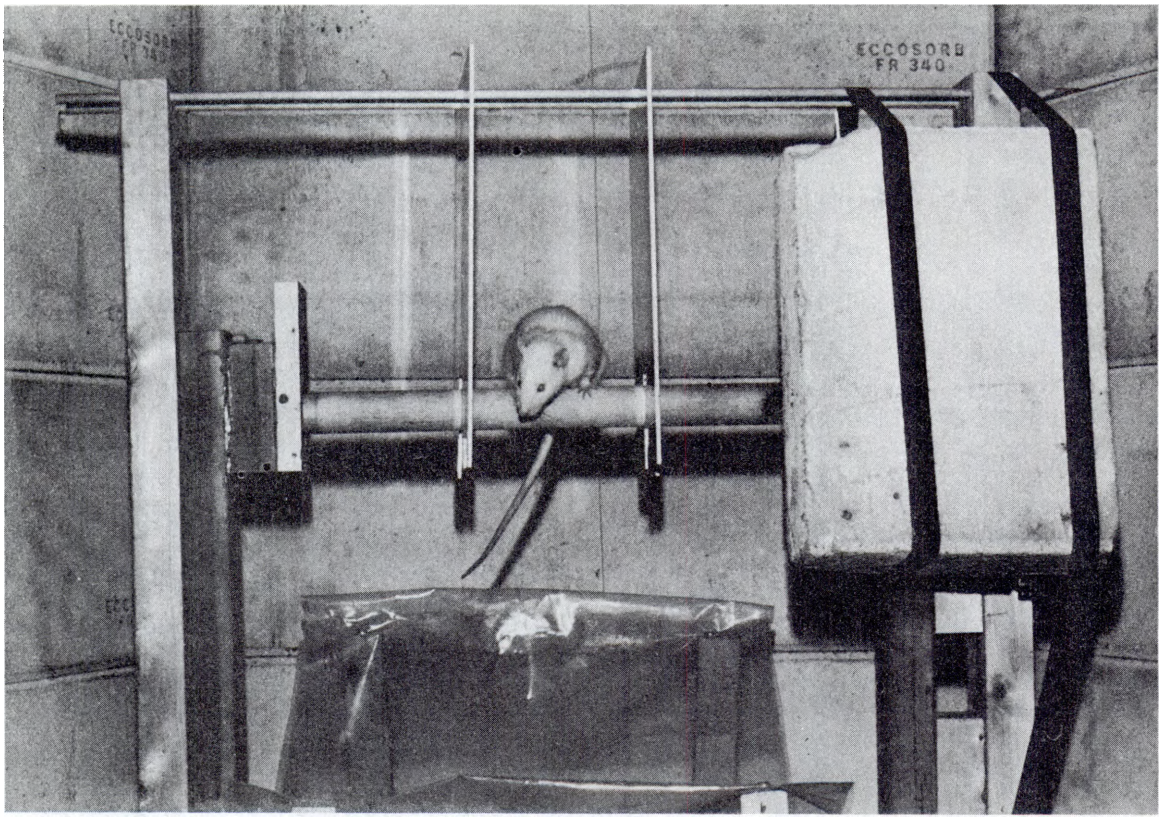
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Figure 5

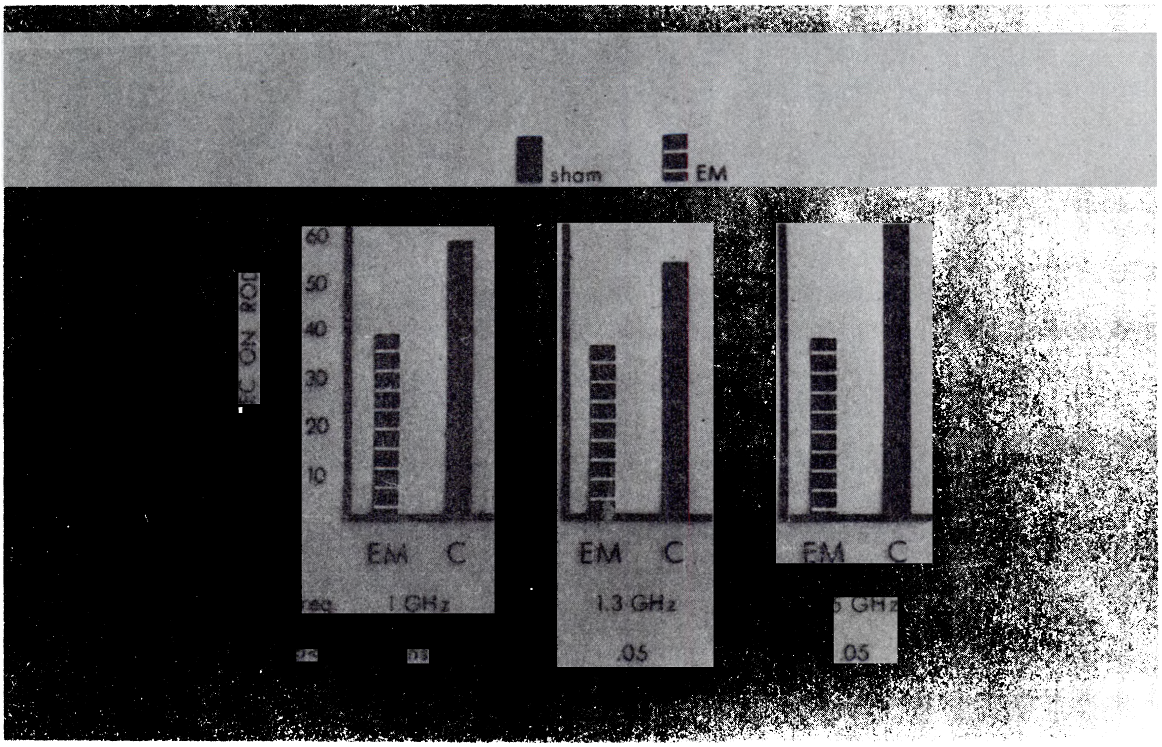

Figure 6

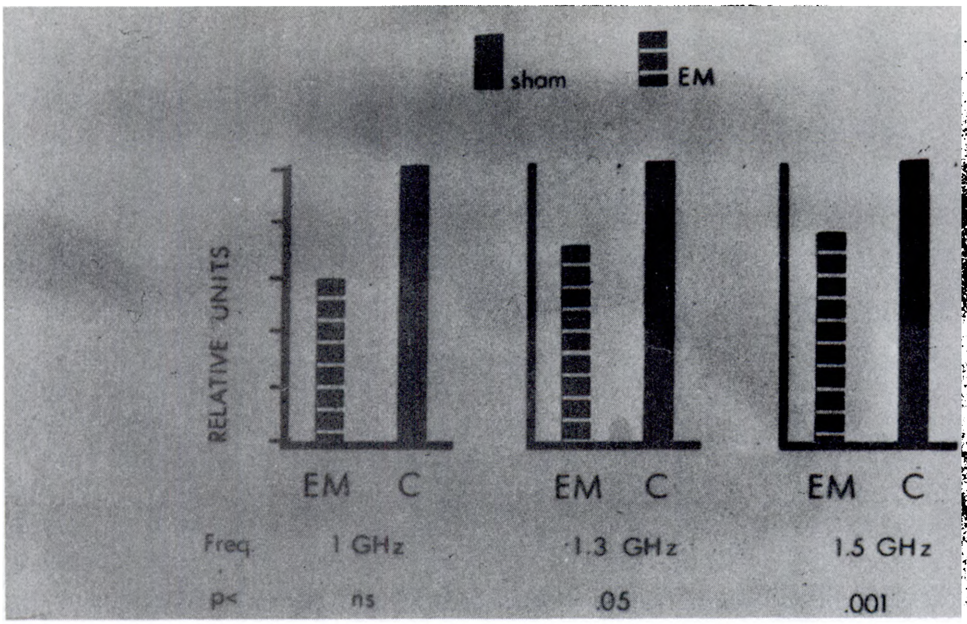

Figure 7

Results

In this experiment, it was found that there were no significant differences between experimental and control means. It was also found that there were no significant differences in the variances. The trends suggest that there is a minimal effect that could possibly be teased out by using large groups; but given the pertubations in the EM energy field that are caused by the presence of the animals, it is felt that we have a reasonable approximation to thresholds for these particular EM energy parameters.

Discussion

Pulse modulated electromagnetic energy has an adverse effect on motor coordination or balance as defined by use of the rotating rod test. The effect occurs at rather low power densities as this is usually defined in biological experiments. The threshold for the effect, with the energy parameters used, is a peak power between 0.4 mW/cm^2 and 2.8 mW/cm^2. The measurements given, although exact, are considered to be correct within an order of magnitude because of the perturbing effect of the animal and any apparatus in the EM field.

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FREE-OPERANT AVOIDANCE AND ESCAPE FROM MICROWAVE RADIATION

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ABSTRACT

Previous research has established that exposure to microwave radiation can effect both innate and acquired behaviors. The purpose of the present investigation was to determine the stimulus properties of microwaves in a free-operant avoidance-escape paradigm. Subjects were CF1 male mice weighing 35-42 g. Two groups of subjects were employed. Experimental subjects received microwaves (2.45 GHz CW) plus tone and sham subjects received tone only. During the session, all subjects could make responses by interrupting a light beam which passed through the animal holder. If no response was made, then the tone remained on for the entire session. Experimental subjects always received microwaves paired with the tone. If the subjects responded during the tone, it was terminated and remained off for 12 seconds. This was considered an escape response. If the subjects responded during this tone-off period, each response would delay the onset of tone for 12 seconds. This was considered an avoidance response. The data show a clear difference in response patterns between experimental and control subjects both in frequency and variability of responding. Furthermore experimental subjects could be categorized by their response patterns into escape animals, avoidance animals, and mixed response animals. For a given subject his response pattern remained consistent across experimental sessions. These data show that microwave radiation can serve as a noxious stimulus which will maintain an active instrumental avoidance or escape behavior over repeated experimental sessions.

INTRODUCTION

Microwave irradiation of a number of organisms and suborganismic systems have shown that microwaves can produce a variety of biological effects (1,2,3). In addition to the biological effects that result from microwave exposure a number of reports have provided data that also show behavioral changes. Korbel (4) reported alterations in the spontaneous activity of rats. When exposed to microwaves, there was an initial increase in activity followed by a subsequent reduction. However, Roberti et al (5) found no change in activity levels of animals exposed for 185 hours. Frey and Spector (6) reported changes in agressive behavior during microwave exposure. In addition to these innate behaviors, studies have been reported of microwave induced changes in instrumental or operant behavioral tests. For instance, Cunitz, Galloway, and Berman (7) reported behavioral suppression of monkeys responding in an alertness task. Thomas et al (8) found alteration in counting efficiency of rats exposed to 2.45 GHz CW radiation, but no changes in overall response rates or response variability. Justensen and King (9) have reported decreases in response rate of animals working on a fixed ratio schedule when exposed to microwaves. These lower rates appeared to be caused by periods where there was a cessation of responding rather than an overall decreased rate of responding.

Some of these behavioral studies appear to indicate that microwaves may be aversive to an animal. In two previous studies, Monahan and Ho (10,11) were able to show that an animal exposed to microwaves will adopt a behavior to reduce absorption of energy if the exposure level is above a threshold value. Furthermore this threshold value is dependent upon factors other than the radiation itself. These studies strongly suggest that microwave radiation can serve as a noxious simulus to an animal. The present study was designed to further test this hypothesis by determining if 2.45 GHz CW radiation could control operant responses in exposed subjects. We tried to answer three very basic questions relative to the stimulus properties of microwaves:

1. Could any differences be seen in response rates between irradiated and sham-irradiated subjects?
2. When microwaves are used as a noxious stimulus will they maintain an operant response rate over repeated exposures?
3. If microwaves do serve as a noxious stimulus, will the subjects avoid the irradiation or will they escape the radiation when given the choice?

METHOD

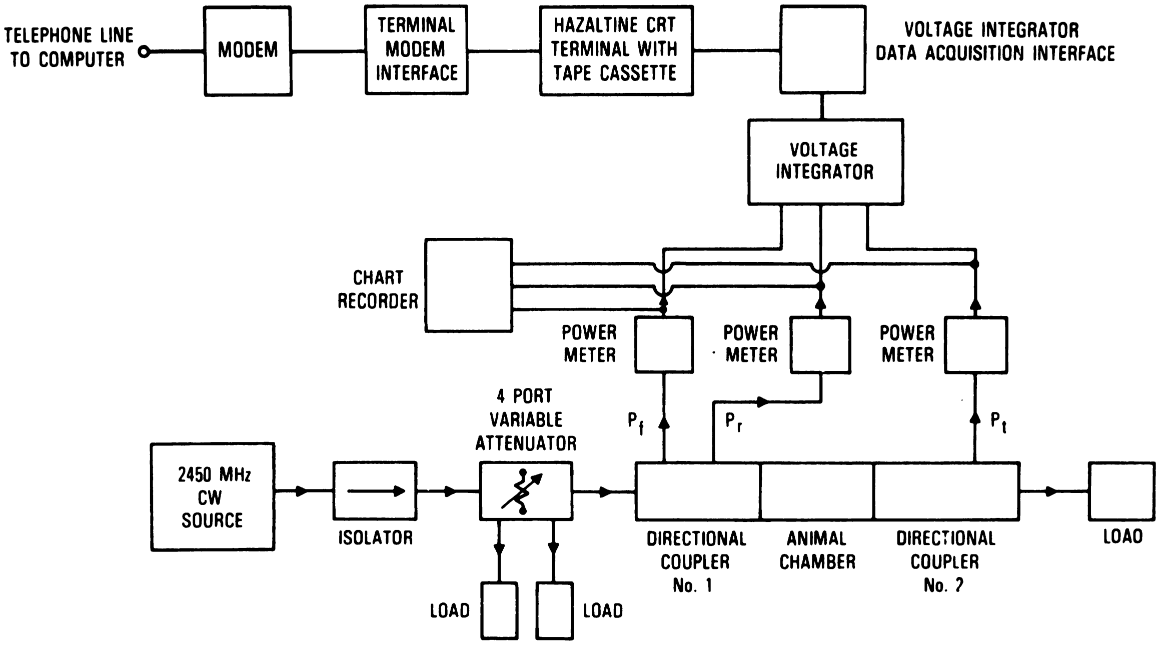
Experimental subjects were all CF1 mice ranging in weight from 35 to 42 g. Throughout the experiment, they were housed in individual cages in the BRH animal colony with free access to food and water. The daily lighting schedule consisted of 12 hours of light and 12 hours of dark.

The irradiation apparatus has been described previously by Ho, Ginns, and Christman (12). It consisted of a generator capable of producing up to 100 W CW power at 2.45 GHz. The output from the generator was fed through a four-port variable attenuator into the animal chamber (Fig. 1). The waveguide unit was a WR 430 waveguide 10.9 x 5.5 cm and operated in the TE_{10} mode. The forward, reflected, and transmitted power were measured through coaxial directional couplers by power meters, and the waveguide terminated in a resistive load. The entire irradiation apparatus was housed within an environmentally controlled chamber that permitted regulation of temperature, and humidity.

The center section of the waveguide was held together with quick release clamps that permitted rapid placement and removal of the animal holder. This holder was constructed of 2.0 mm black, opaque Plexiglas with holes in either end to permit air circulation, and a sliding top for insertion of the animal (Fig. 2). The outside dimensions of the holder were 26.7 x 5.4 x 3.2 cm and this size permitted the animal to move about freely. Two holes were placed in the sides of the holder 1.5 cm from the floor and 0.75 cm from the end. This permitted a light to shine through the box to a photoreceptor. Both the light bulb and photoreceptor were external to the waveguide and light passed through two 0.635 cm holes in the waveguide.

The behavioral control system was located outside the environmental chamber. This programming equipment consisted of K logic modules supplied by Digital Equipment Corp. Responses and microwave exposure durations were recorded on impulse counters, and also on a Gerbrands cumulative recorder.

Because the animals were free to move about within the holder, their absorbed dose could vary. Therefore, we integrated the readings from the power meters with respect to time to arrive at an integral dose rate. Average absorbed dose rate (mW/g) can be defined as the total energy absorbed by the


TELEPHONE LINE TO COMPUTER
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TERMINAL MODEM INTERFACE
HAZALTINE CRT TERMINAL WITH TAPE CASSETTE
VOLTAGE INTEGRATOR DATA ACQUISITION INTERFACE
VOLTAGE INTEGRATOR
CHART RECORDER
POWER METER
POWER METER
POWER METER
4 PORT VARIABLE ATTENUATOR
P_f
P_r
P_t
2450 MHz CW SOURCE
ISOLATOR
DIRECTIONAL COUPLER No. 1
ANIMAL CHAMBER
DIRECTIONAL COUPLER No. 2
LOAD
LOAD
LOAD

Figure 1. Block diagram of the environmentally controlled 2.45 GHz waveguide irradiation apparatus.

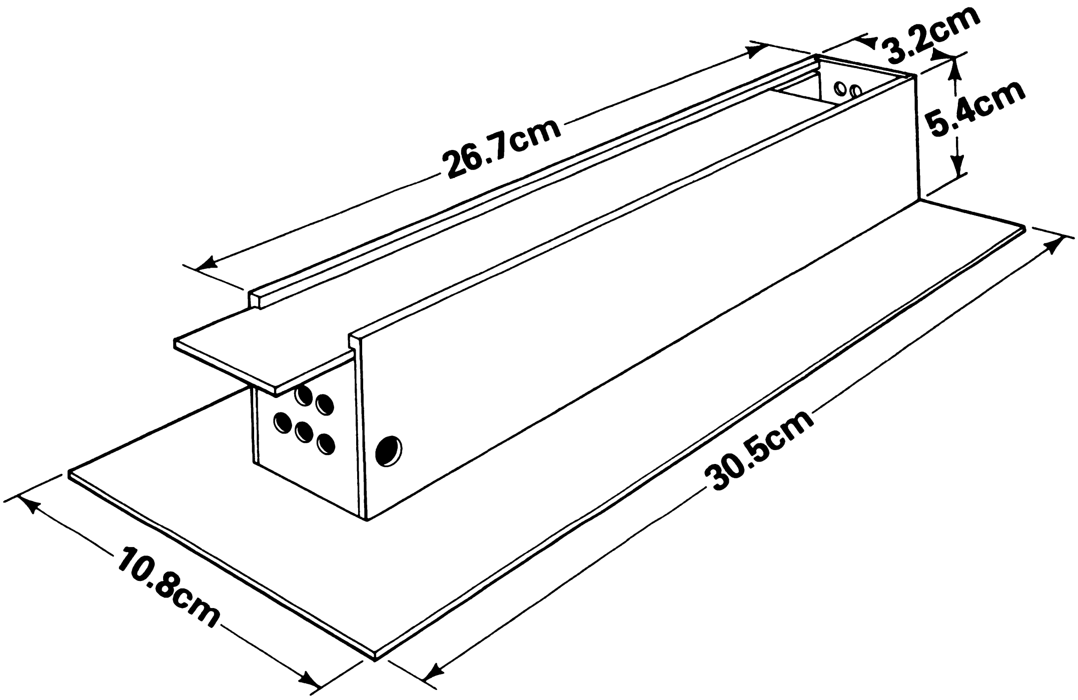
3.2cm
5.4cm
26.7cm
30.5cm
10.8cm

Figure 2. Schematic drawing of the animal holder showing lateral location of holes for light beam.

subject over a given time period, divided by the time interval, and the weight of the animal. Percent absorption was defined as the percent of the forward power absorbed averaged over a given time period.

PROCEDURE

Experimental sessions were conducted daily and lasted 30 minutes. Each subject was placed in the animal holder that was then placed in the waveguide. Environmental conditions were 24 ± 0.5 C and 40-50 percent relative humidity. A response consisted of the interruption of the light beam passing through the animal holder and a relay mounted just outside the waveguide was actuated with each response to provide response fed back to the animal.

During the experimental session a 2900 Hz tone (Sonalert SNP428) was paired with 2.45 GHz CW radiation with a forward power level of 2.7 W. In our experimental procedure, there are three possibilities or combinations that can occur:

1. No response - the microwaves and tone will come on 12 seconds after the session begins and remain as long as no response is made.

2. Escape response - once the microwaves and tone are initiated, a response will terminate them and they will remain off for 12 seconds or until an avoidance response is made.

3. Avoidance response - during the interval when no microwaves or tone are present, each response will reset a 12-second timer, thus delaying the onset of microwaves and tone for an additional 12 seconds.

Two groups of animals were run in the procedure, i.e. an experimental group (N=5) and a sham group (N=3) that had the microwave generator turned off. Thus, this later group received only tone presentations and the only consequence of responses was to escape or avoid the tone. Since no visual observation of the animals was possible, no response shaping was performed. All subjects were placed into the experimental situation without any prior training.

RESULTS

All subjects, both experimental and sham-irradiated, showed some responding in this behavioral situation. However, when we compare the mean tone-on times (Fig. 3) there is a very clearcut difference between the two groups. Subjects 1 through 5 who had microwaves paired with tone had mean on-times of less than 20 seconds. Subjects 6,7, and 8 received no microwaves and had significantly higher on-times and showed much larger variability between sessions than the exposed subjects (Fig. 4). Whenever the experimental animals were exposed to microwaves, they terminate the radiation by a response within 20 seconds following onset.

All experimental subjects were exposed to a forward power level of 2.7 watts. This resulted in an average absorbed dose rate of 46 mW/g and a mean percent absorption of 62 percent. However, it should be noted that the duration of exposure to microwaves was contingent upon the subjects' behavior and therefore not under the control of the experimenter. Because of this contingency the total exposure duration during a session varied for each subject. The individual exposure segments within a session and the time intervals between exposures were also variable (Fig. 4).

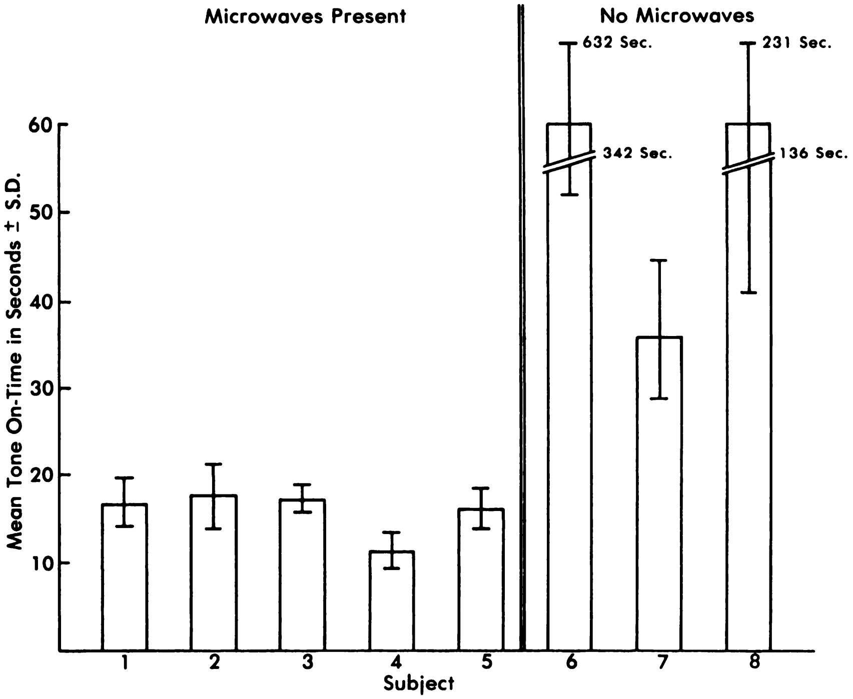

Figure 3. Graph of mean tone on-time for each subject averaged over eight experimental sessions.

The consequences of a subject's responses in this procedure were either escape from microwave radiation once it has been initiated or avoidance of the onset of the radiation. Figure 5 represents the percent of escape responses when compared to the total number of responses. Subject 3 shows nearly 30 percent of all his responses are escape responses. It is therefore possible to categorize his behavior as primarily escape. As seen in figure 4, the avoidance responses which he typically makes are due to short bursts of responses following the escape response, i.e., he makes two or three responses in rapid succession and only the first of these is recorded as an escape response.

Subjects 1 and 2 (Fig. 5) have about 15 percent escape responses. This suggests that these subjects are mixing their responses between escape and avoidance and, consequently, it is difficult to categorize them as either escape or avoidance animals. In figure 4, their typical cumulative records indicate escape behavior intermingled with bursts of avoidance responding. This behavior was exhibited consistently by these subjects across the experimental sessions that we analyzed. There did not appear to be any preference on the subject's part for a specific response pattern.

Subjects 4 and 5 (Fig. 5) show less than 10 percent escape responses. This suggests that these subjects are avoiding the onset of microwave radiation. While it is true that they did permit the radiation to come on periodically, these onsets were typically followed by prolonged periods of avoidance responding. This can be seen in figure 5 which shows typical cumulative records for these two subjects. It should also be noted that the average on-time for these subjects was generally less than that observed with an escape animal such as subject 3.

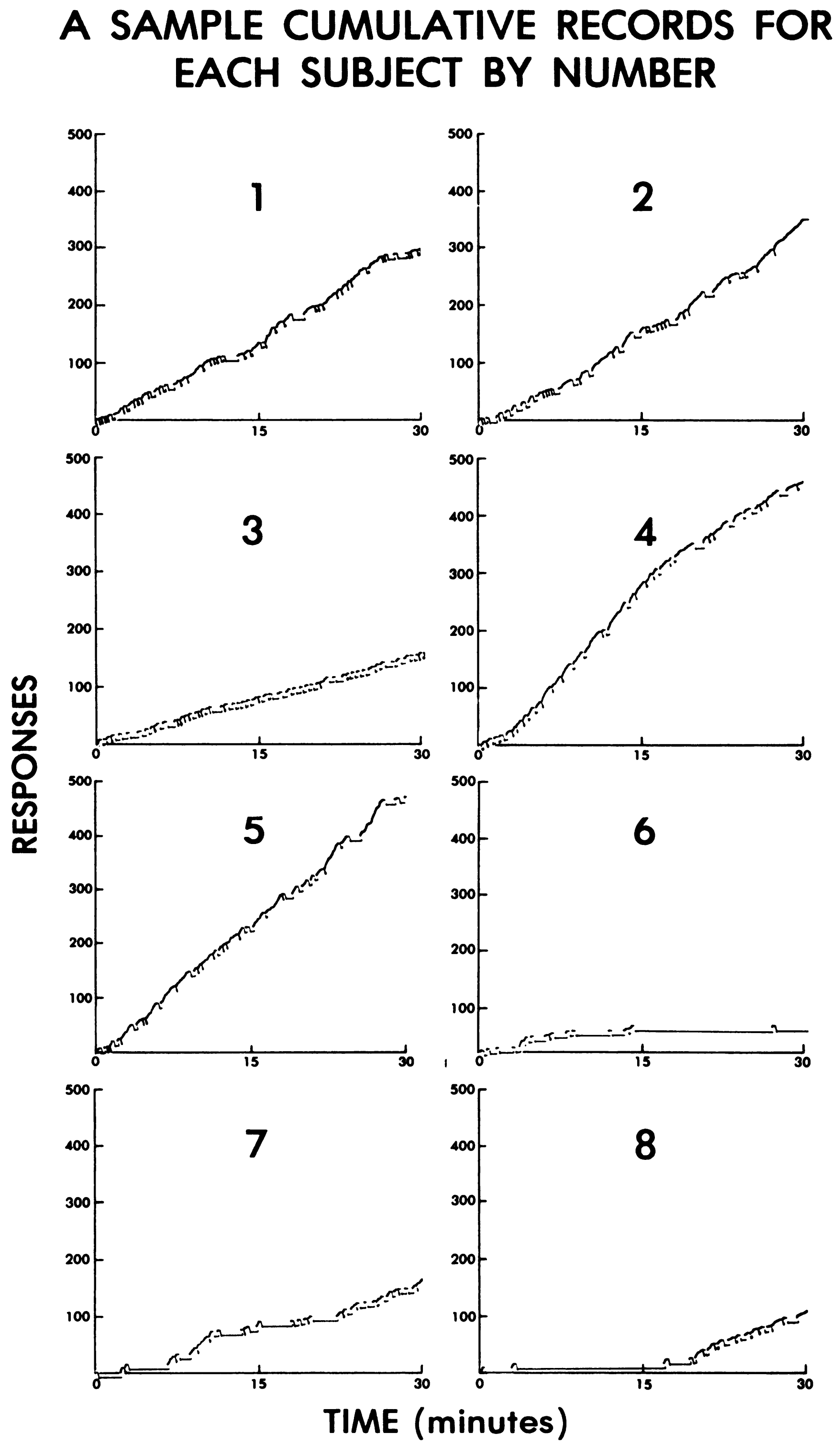

Figure 4. Graph of percent escape responses for each subject averaged over eight experimental sessions.

Figure 6 is a representation of the number of avoidance responses, a subject made for each escape response. Subject 3 stands out as primarily an escape animal, showing only two avoidance responses for each escape. Subjects 4 and 5 clearly demonstrate a preponderance of avoidance responses. For each escape response, these subjects are making approximately 15 avoidance responses. In the case of subjects 1 and 2 the data are not as obvious as in the preceding subjects. They have approximately six avoidance responses for each escape response. However, when the cumulative records are examined, it can be seen that they are vascillating between avoidance and escape responses with short periods of each.

DISCUSSION

The results presented here confirm and extend the previously reported observations (10,11) that microwave radiation can serve as an aversive stimulus. This data shows that a subject will perform an operant response to escape or avoid microwave radiation.

Frey and Feld (13) have reported that rats will avoid pulse-modulated 1.2 GHz radiation by spending more time in the shielded half of a shuttle box. However, they failed to observe any avoidance behavior when animals were exposed to continuous wave radiation, even at higher power levels. Subsequently, Hunt et al. (14) reported similar findings. Rats exposed contingently to 2.88 GHz pulsed radiation displayed escape behavior after about 15 minutes. Both of these studies employed pulsed radiation and the response measured was a passive type i.e., the animal, to escape or avoid the radiation, had to remain in the shielded half of a shuttle box. The present results extend the above findings to another microwave frequency and to continuous wave radiation. They further demonstrate that microwave radiation can serve as a noxious stimulus for maintaining a response that actively escapes or avoids radiation. In the present procedure a response consisted of the interruption of a light beam in order for the subject to avoid or escape the radiation.

The data presented above demonstrate that it is possible to distinguish between irradiated and sham-irradiated subjects on the basis of operant response rates. Sham-irradiated subjects tend to have very low response rates, or only occasional bursts of responding. Irradiated subjects whose responses either permit them to avoid or escape the radiation will work consistently throughout the experimental session. Furthermore, when the mean interresponse time is examined, the sham animals have a much higher interresponse time. These differences, both in response rates and interresponse times, thus serve as reliable indicators of the irradiation condition in the experimental procedure.

The present data show that microwave radiation will function as a stimulus and that it maintains consistent avoidance or escape response rates over successive experimental sessions. While sham-irradiated subjects have either very low or inconsistent respose rates, the subjects exposed to radiation will consistently work to lower their exposure.

In the present experimental situation, the subjects, if they respond, have three choices: avoid, escape or mix their responses. The data show that a given animal will adopt one of these three possible behaviors and then consistently maintain this behavior. For instance, an animal which escapes from the radiation does not switch to an avoidance response pattern but rather maintains its escape behavior pattern. The determinants that produce a specific behavior in one subject as opposed to one of the other possibilities is uncertain at the present time.

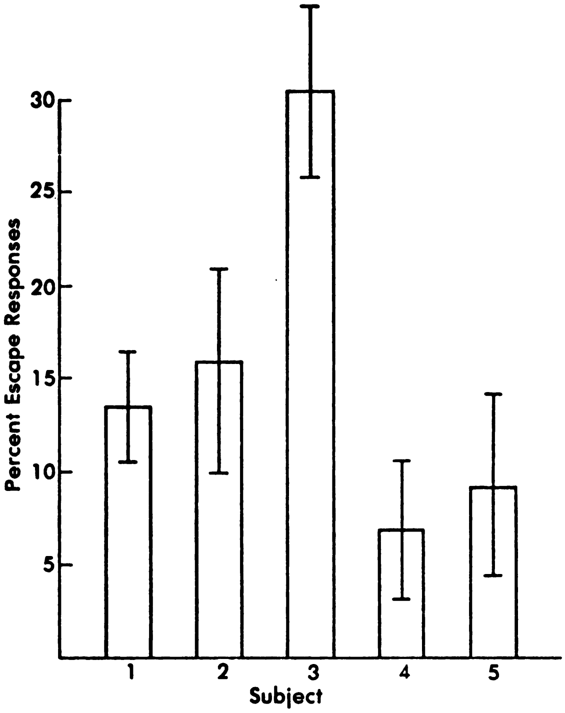

Figure 5. Typical cumulative records for each of the eight subjects. Each downward offset of the pen indicates tone on or tone plus microwaves on. Subjects 1 through 5 are experimental. Subjects 6, 7, and 8 are sham exposed.

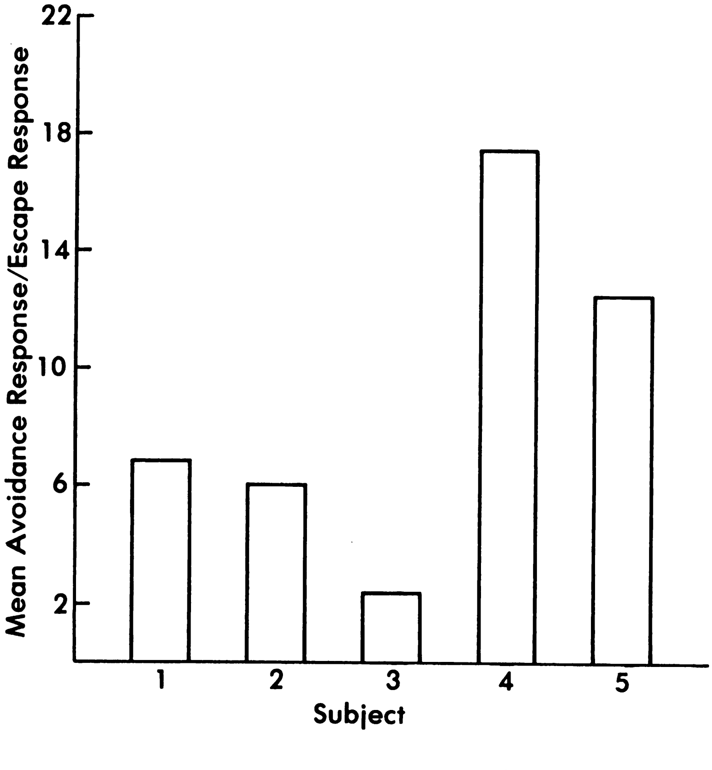

Figure 6. Graph of mean number of avoidance responses for each escape response for each experimental subject averaged over eight experimental sessions.

The specific behavior that an animal develops in the presence of microwave radiation appears to be dependent upon the available contingencies and not the radiation itself. Some of the behaviors which have been observed are avoidance and escape, both active and passive (13,14) and reduction of energy absorption by orientation of the body relative to the incident radiation (10). These behaviors have been observed over a variety of frequencies, exposure levels, and environmental conditions. All of these findings tend to confirm the hypothesis that microwave radiation can serve as a noxious stimulus. Consequently subjects will try to reduce or avoid absorption of this energy if it is above a threshold value. While the exact mechanism underlying these observed behaviors is still uncertain, the use of this type of approach is continuing to further our knowledge about the interaction of microwaves and living organisms.

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OPEN DISCUSSION

DR. SESSION: I would like to know a little bit about the nature of the unconditioned stimulus that the animals actually are avoiding or escaping. Could you translate this into language; such as how long would it take the animal if he did not escape the irradiation to go into obvious heat stress, or what would be the colonic temperature of the animal after a certain level or duration of exposure?

MR. MONAHAN: I might relate this to some previous work that we did. It was also an avoidance study, but it was an avoidance study where the animal could only lower his absorption by orienting his body rather than totally avoiding or escaping. In that study, at approximately the same milliwatt per gram dose, we observed temperature increases under continuous exposure for 15 minutes. The increases were of the magnitude of approximately half a degree rise (Centigrade). Although we did not take colonic temperatures in this experiment, I would assume that if the animal did not respond for the duration of the session, there would be a rectal temperature increase. However, all of the animals responded in this situation, including the sham animals. There is just a much larger difference in rates.

MRS. FERRI: Have you ever put any of these animals back in for a second session to determine whether their response is different in a repetitive type situation?

MR. MONHANAN: Yes. Perhaps I did not make that clear in my talk. The graphs that I showed you were the means of eight sessions. These animals were, in fact, run repetitively. They were put into the situation, and obviously there is some learning involved; so they were run for a number of sessions until their behavior stabilized. What I tried to do in putting this data together was to interpret the behavior after it had reached this stabilized condition. The sessions that I showed you were after approximately 15 or 20 previous sessions.

DR. PHILLIPS: I have some data which may be relevant to this. We have exposed 25 gram mice to 50 milliwatts per square centimeter. The temperature rise over a 2-1/2 minute period is about a half degree. I have also made some absorbed dose measurements from these animals, and the absorbed dose is very comparable to what you used here--about 45 to 50 milliwatts per gram. Do you plan on replicating these studies at lower values?

MR. MONAHAN: Yes, I do. We have a number of studies planned and that is one of them. I might ask you, Dr. Phillips, on the exposures that you gave, did you have air flow?

DR. PHILLIPS: Yes. Exposure took place in an anechoic chamber. We did not have the precision you had here, obviously. The chamber temperatures were maintained at about 74 . The air flow rate in the chamber was around five changes an hour, but there is nothing said about uniformity. We have the venting system, so it is consistent but not necessarily uniform throughout the anechoic chamber itself.

MR. MONAHAN: Yes. I might mention that in our system we do have a fairly high air flow rate, so this does provide opportunity for the animal to cool itself.

DR. SHORE: What is the lowest level of exposure that is associated with any type of avoidant response in your studies?

MR. MONAHAN: In this particular study we were operating at 24 C temperature. We picked a level--approximately 46 milliwatts per gram, that we knew would give us avoidance from previous studies. However, on a previous study with an increase in temperature to 95 F, which is a warm summer day, we got avoidance levels down to 0.6 milliwatts per gram.

MICROWAVE ABSORPTION AND TASTE AVERSION AS A FUNCTION OF 915 MHz RADIATION

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Division of Biological Effects
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ABSTRACT

Thirty-two rats were divided into four subgroups and exposed to 915 CW radiation at different intensities immediately following 15 minutes access to a 10 percent sucrose solution. Absorption of microwave energy was relatively unchanged throughout a 15-minute exposure to forward power of 5.0 W. Microwave absorption progressively decreased during exposure at forward power levels of 9.1 and 19.0 W. Each subject was again given access to sucrose 24 hours later, with no indication of a conditioned taste aversion.

INTRODUCTION

A variety of behavioral conditioning procedures have been used to assess the hazardous effects of radiation upon behaving organisms (1,2,3). Our studies section within BRH has used this rather broad based approach to examine the potentially deleterious effects of microwave exposure upon functional behaviors. Our efforts are basically keyed to particular experimental definitions of disruptive or aversive effects of physical stimuli. For example, functional damage may be defined as a general suppression or dimunition of normal, ongoing behavior, and we have examined the effects of microwave exposure upon ongoing operant or instrumental behavior (4) as well as upon Pavlovian or classical conditioned responses (5). Secondly, Monahan and Ho (6,7) have also conducted a series of experiments to determine whether microwave radiation may directly control escape or avoidance behavior commonly reported with other aversive events. The present experiment was based upon a third potential characteristic, and used a standard conditioned aversion procedure to examine acquired aversions to stimuli associated with noxious agents such as shock, x rays, pharmacological agents, etc., (8,9). Specifically, rats were given accesss to a 10 percent sucrose solution, and then immediately exposed to microwave radiation. The potentially conditioned aversion to sucrose was then assessed with a one bottle preference test relative to sham controls. Quite recently, Hjersen, Phillips, and Lovely (10) used a similar procedure and reported no acquired taste aversion to a saccharin solution. The present experiment was also designed to systematically replicate the unconditioned attenuation of microwave absorption previously reported (6) and employed a different species (rats versus mice) and different radiation frequency (915 versus 2450 megahertz).

MATERIALS AND METHODS

Thirty-two experimentally-naive male Sprague-Dawley rats served as subjects. Each subject was individually housed with food freely available and with water available 15 minutes per day. The light-dark cycle consisted of 12 hours each. In the experimental phase, each subject was given access to a sucrose solution rather than water during the 15-minute period.

Our general microwave exposure technique has been previously described using a 2450 MHz CW system (5,6). The present experiment, however, employed a 915 MHz source and appropriate waveguide with a cross section of 24.8 by 12.4 cm (11). The average absorbed dose rate (mW/g) was defined as the total energy absorbed by the subject over a given time period divided by the time period and the weight of the animal. Percent absorption was defined as the percent of the forward power absorbed averaged over a given time period.

Within the waveguide, the animals are restrained in a 23 by 21 by 9 cm Plexiglas holder, with walls 3.0 mm thick. Two opposite walls are a grid of plastic strips to allow airflow through the holder. Air is forced through the waveguide at 38 l/min. The temperature of the exhausted air ranged between 23 and 26° C and the room humidity was 40-50 percent during experimental sessions.

On the sucrose microwave pairing day, each subject was given access to a 10 percent (by weight) solution of sucrose for 15 minutes. The subjects were then arbitrarily divided into four subgroups. Group four was a sham control group, with each subject placed in a dummy waveguide chamber for 15 minutes immediately following sucrose presentation. Subjects in groups 1, 2, and 3 were placed in the waveguide and exposed to microwaves with forward power of 5.0, 9.1 and 19.0 watts, respectively. The absorbed dose averaged over the 15-minute exposure was 7 (Group one) 10, (Group two) and 17 mW/g (Group three). The average absorbed dose rate and percent absorption was also recorded at each 5-minute interval during the irradiation. Each subject was immediately returned to the home case following microwave exposure. After a 24-hour period, each subject was given a one-bottle preference test. The 10 percent sucrose solution was again available for 15 minutes, and the amount consumed was compared to the liquid consumed on the previous day.

RESULTS

Figure 1 presents the absolute amount of microwave energy absorbed during successive 5-minute intervals for each radiation exposure group. The percent of applied radiation actually absorbed remained relatively constant throughout the 15-minute exposure for the subjects exposed to 5.0 watts forward power (lower panel of Fig. 1). However, the percent absorption progressively decreased across successive 5-minute exposure intervals when the applied power was 9.1 W (middle panel, Fig. 1). Similarly, percent absorption monotonically decreased at the highest forward power used in the present experiment i.e., 19.0 W (upper panel, Fig. 1). The average absorbed dose rate in successive 5-minute intervals in mW/g were 7.1, 7.1, 7.1, at 5.0 watts forward power 10.5, 9.5, 8.7, at 9.1 watts forward power, and 18.5, 16.9, 16.5, at 19.0 watts forward power, respectively. The experimental results are replotted as relative rather than absolute absorption changes in figure 2. The absorption during the second and third 5-minute intervals are expressed as negative or positive changes relative to the first 5 minutes of exposure. The absorption during the second 5 minutes relative to the first 5 minutes was clearly decreased at the two highest forward power levels. The decremental effect was still more exaggerated during the third 5-minute interval. The change in absorption was generally a U-shaped function of forward power across experimental groups.

Lastly, figure 3 compares the amount of sucrose solution consumed before and after pairing with microwave for each group of subjects. The data within and across groups give little evidence of a conditioned aversion to sucrose following microwave pairing.

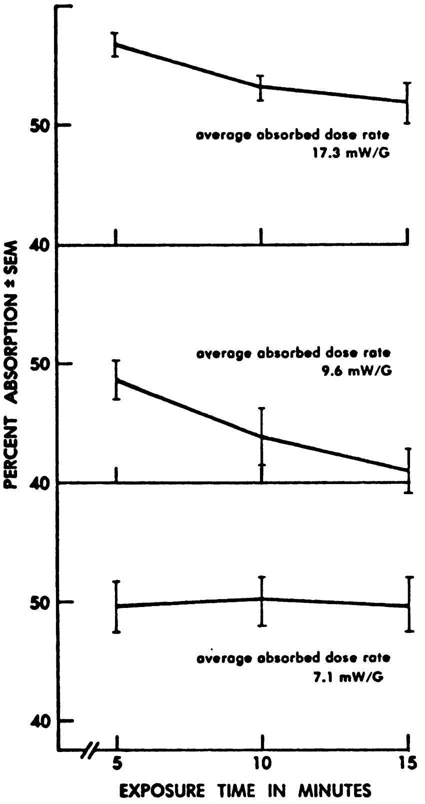

Figure 1. Percent absorption of 915 MHz CW radiation by rats at three average absorbed dose rates.

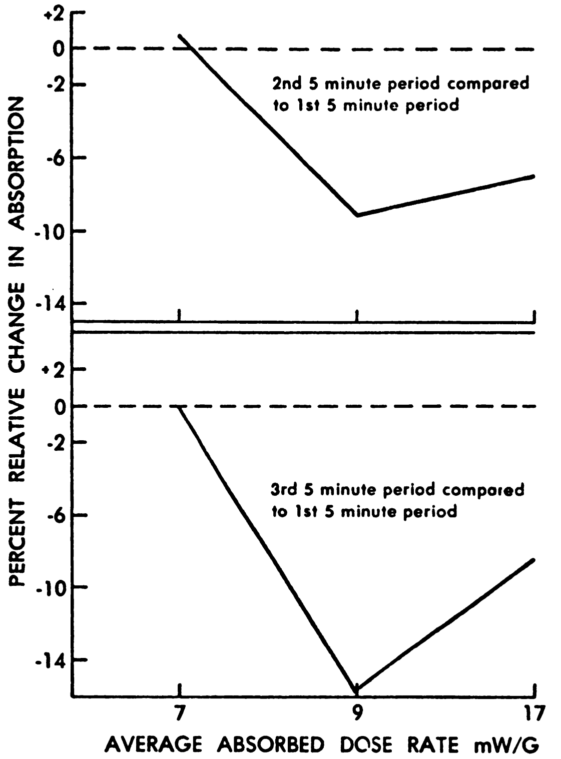

Figure 2. Relative percent change in average absorbed dose rates during a 15-minute exposure to 915 MHz CW radiation.

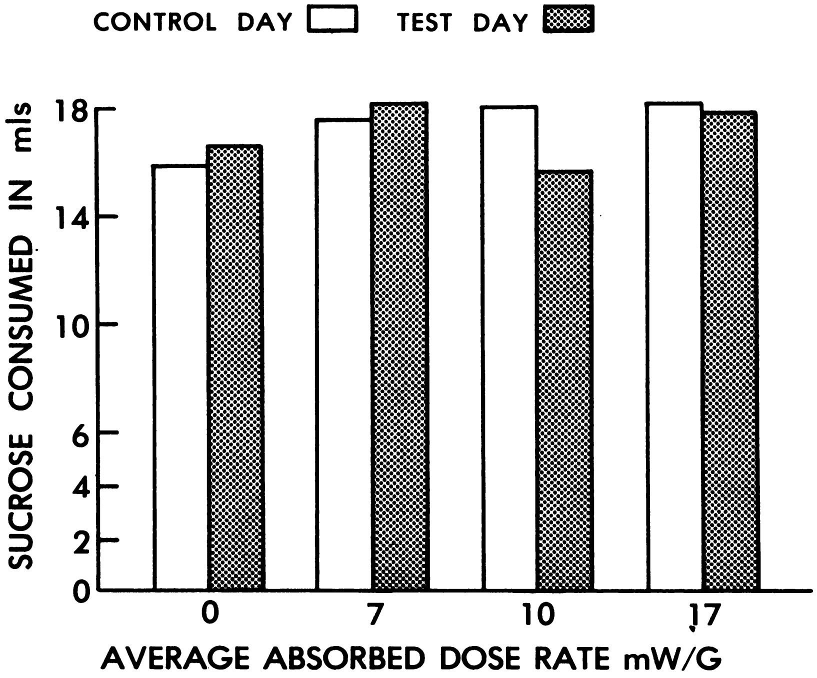

Figure 3. Amount of 10 percent sucrose consumed on control and test days by rats exposed to 915 MHz CW radiation.

DISCUSSION

The results of the present experiment clearly suggest a temporal pattern in the absorption of microwave energy by living organisms. Radiation absorption was a systematic function of forward power, with relatively unchanged absorption at forward power of 5.0 watts but progressively decreasing absorption throughout exposure with higher forward power levels of 9.1 and 19.0 watts. The absorption function does not seem to be linear, however, with the maximal relative charge of 16 percent observed in group two, and smaller changes of about 8 percent for the subjects in group three exposed to a relatively higher forward power of 19.0 W. The absorption pattern across groups is then at least suggestive of a U-shaped function of applied forward power. Conversely, the subjects did not acquire a taste aversion to the sucrose solution paired with microwave radiation. The amount of sucrose consumed following microwave exposure was independent of radiation intensity, and unchanged relative to sucrose consumption on the preceding day (within-subject comparison) or to sham controls (between-group comparison).

The present findings of attenuated microwave absorption without an acquired taste aversion would seem to be consistent with previous experimental results. Hjerson, Phillips and Lovely (10) reported an absence of conditioned aversion to saccharin paired with pulsed microwave energy at 2.88 GHz. The present results would systematically extend the previous findings to include sucrose solution paired with continuous wave irradiation at 915 MHz. On the other hand, Monahan and Ho (6) have reported similar decreases in energy absorption with mice exposed to 2450 MHz radiation. The temporal pattern of energy absorption by mice was similarly related to forward power levels, and may also be conditional upon other factors, such as ambient temperature (7). In combination, the present and previous results suggest that the calculated energy absorption may not be a complete characterization of microwave effects when averaged across exposure duration. Rather, the data suggest a systematic alteration in energy absorption within exposure conditions, which may require a sequential analysis of time and intensity exposure parameters.

Morever, the microwave attenuation effect may be related to elicited orienting behavior within the microwave field which minimize energy absorption and maximize energy reflection (6,7). This behavioral analysis then suggests that actual absorbed microwave energy may be a dependent variable characterizing the subjects behavior, rather than an independent variable exclusively defining microwave properties. A number of investigators have in fact reported that microwave energy may control or elicit avoidance or escape behavior (12, 13, 14). In contrast, the superimposed effects of microwave radiation upon behaviors controlled by classical and operant reinforcement schedules are less clear-cut (15, 16, 17).

CONCLUSION

The apparent interaction or feedback between behavior and microwave energy would suggest that responses which attenuate or avoid energy absorption may be potentially important and sensitive determinants of microwave exposure effects.

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OPEN DISCUSSION

DR. SESSION: I might ask you the same question that I asked Mr. Monahan, in terms of colonic temperature measurements after exposure. Have you taken a look at the potential colonic temperature increases after the 50 minute exposure in your highest group?

DR. HENTON: Yes, we did initially. However, we immediately ran into a problem because the procedure for taking colonic temperatures was aversive. We found conditioned taste aversions in the sham animals as well as in the experimental animals.

DR. SESSION: I am not too surprised at that, but this can be habituated out. Animals could get used to hanging, if it did not kill them.

DR. HENTON: That is true. We would guess that the temperature rise, based on previous data that Jack has here, would be less than 2° at the highest power level that we use. Would you agree with that?

DR. SESSION: Approximately no higher than 39° C?

DR. HENTON: I would think so.

DR. SESSION: That is interesting in that at Walter Reed we have been performing similar experiments--looking at the conditioned taste aversion in a free field situation with 986 megahertz. We found that you can pick up an aversion, but not until you get at least a 2° to 3° C increase in colonic temperature as a measure of absorption. It is clear that heat exhaustion is a sufficient motivational stimulus to motivate learning the taste aversion. We have similarly seen no aversions at lower levels, even though we have seen temperature increases and beginning signs of heat exhaustion.

DR. HENTON: I think that it seems to be a very consistent pattern. I had initial high hopes that this taste aversion procedure, which had been very nicely developed in the ionizing radiation field, would prove to be rather successful with microwaves. But I think that Lovely has done this work, and the work that you mentioned at Walter Reed tends to present a consistent picture of this pattern.

DR. SESSION: I think it speaks to the difference in the motivational substrate. The way I look at it, the nature of the aversive conditions with microwave power are certainly there when you are in danger of pushing the animal over his ability to thermoregulate. However, as soon as you take the microwave energy away, if the animal is still living, the motivational substrate goes away with something like ionizing radiation or any other so-called illness inducing agent. The aversive conditions, physiologically, can be around for hours and hours, especially ionizing radiation where radiation sickness does not start for at least 1/2 an hour to 1 hour and can last for a great deal of time. I think, perhaps, the length of time that you maintain the animal in a condition of physiological discomfort is an important variable. I would expect that if you took some of your higher power level groups and ran them out longer--1 hour, 2 hours--you might increase your chances of picking up a behavioral effect.

DR. HENTON: There are a number of parameters here that we could play with. I am not sure that I would agree on this particular parameter, in that you can present shock with 50 or 100 milliseconds.

DR. SESSION: But shock is not an internal stimulus either.

DR. HENTON: Yes, but it is a very brief stimulus.

DR. PHILLIPS: It might give a little perspective, when we talk about absorbed doses and energies, if we related back to what the metabolic rate of a rat is. What does 17.3 milliwatts per gram actually mean? If you look at the metabolic rate of a rat (basal rate)--this is calculated--it runs at about 25 small calories per minute, which is about 4 milliwatts per gram for a 450 gram rat. When you get up to a value like 17.3, you are adding an equivalent of 4 times his metabolic rate to the basal rate, which is a pretty hefty heat load on a rat.

DR. HENTON: Yes, that is true.

BEHAVIORAL PROTOCOLS AND PRELIMINARY OBSERVATIONS OF CHICKS EXPOSED TO WEAK AMPLITUDE MODULATED 450 MHz FIELDS

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ABSTRACT

Experiments with the possible effects of weak ELF fields on behavior of primates have led to conclusions that are relevant to studies of VHF on microwave exposure. These include (1) the notion of frequency specific effects (2) the possibility of voltage windows (3) the importance of duration of exposure, and especially (4) the issue of masking of behavioral effects by external stimulus control intrinsic to the task being used to assay effects. External stimulus control has also been demonstrated to be an important variable in behavioral toxicology and in behavioral studies of brain lesions. Taking these factors into consideration a series of studies has been designed to assay the behavioral effects of weak amplitude modulated VHF in neonatal chicks and wild mallard ducklings. This programmatic approach will be three dimensional: (1) Across power levels, (2) across "biologically relevant" and "non-relevant" frequencies, (3) across increasingly complex time-based schedules of reinforcement. Preliminary studies at 5 mW/cm^2 with amplitude modulation set at 3 Hz or 16 Hz were done with an FT 30 sec (fixed time schedule of reinforcement). Results suggest that simple activity measures are inadequate assays of possible weak effects whereas latency measures are sensitive to the presence of the field.

INTRODUCTION

Background of ELF Behavioral Studies and Implications for EMR Studies in General.

Our primary behavioral work up to this point has been done with primates (**macaca nemestrina**) exposed to weak ELF fields. These studies have led to the formulation of certain guidelines for our present work with weak, amplitude-modulated VHF fields, expecially regarding the selection of an appropriate behavioral assay.

Over the past several years, there have been observational studies (as contrasted with laboratory studies) of the role of weak ELF fields in affecting the survival behavior of certain species. One example of such a study is that of Kalmijn (1) in which he demonstrated that sharks use weak, ELF fields to detect their prey. In an ingenious series of experiments, Kalmijn demonstrated that sharks detected a flat fish buried in sand at the bottom of a large holding tank on the basis of the weak ELF field emitted by the flat fish. Kalmijn estimated this field to be on the order of 0.2 μV/cm modulated by slow respiratory rhythms. When non-electric cues were systematically eliminated by the use of an agar chamber the shark continued to dive immediately to the buried flat fish. However, the introduction of a thick polyethelene film placed around the prey attenuated the electric field and succeeded in confusing the shark. When the natural field of the flat fish was simulated with a 0.4 μV/cm field at 5 Hz, produced by two electrodes buried in sand at the bottom of the tank, the shark dove immediately to the location of the electrodes. In later experiments, Kalmijn observed a voltage window: i.e. the effect was not observed if a substantially higher voltage simulation was used. Special receptors, the ampullae of Lorenzini, were discovered to account for the sharks perception of the weak fields.

Other observational studies have been done on the homing and migration of birds. The study of Keeton (2) is especially interesting. Although this study employed weak magnetic fields rather than electric fields, it is described briefly here because of an important methodological point. Keeton strapped tiny magnets on the backs of homing pigeons and observed that their flight was, consequently, disoriented--but only on cloudy days. He concluded that if the sun were present as a salient cue, the pigeons ignored the presence of the weak magnetic cue. The sensitivity of the pigeons could only be observed when strong external cues guiding their behavior were absent.

New evidence for disruption of migration by weak ELF fields has recently been offered by Williams, Williams, Larkin and Sutherland (3). They have observed that migratory birds showed a deviation in flight direction of 5° to 25° around the Seafarer antenna, when the N-S axis was energized. Indications were that flight direction was rapidly corrected. This field was estimated to be 0.17 V/m rms at 10 meters, perpendicular to the antenna.

These observational studies raise the question of whether they represent merely isolated peculiarities of nature or whether they point towards some fundamental property of nervous systems that extends throughout the animal kingdom, including man.

In 1969 we began a long series of studies, Gavalas, Walter, Hamer and Adey (4), Gavalas Medici and Day-Magdaleno (5), aimed at exploring the possible effects of weak ELF fields on behavior of monkeys in the laboratory. It seemed obvious, at the outset, that low frequency fields at levels of 1-100 V/m p-p, were not likely to produce dramatic, sudden onset, behavioral changes. (Fields at those levels are routinely encountered in the home around 60 Hz devices.) It was taken as a challenge to find a (1) highly sensitive but reliable behavioral assay that could, in principal, (2) detect thresholds and would allow (3) long exposure durations. For example, it seemed vain to think that five minutes of exposure to a 45 Hz field at 10 V/m would affect any behavior at all. A final requirement, based on repeated references in the literature to reaction time, circadian rhythms, etc., was that the task involve some kind of (4) "timing" behavior. These four requirements all seemed to be met by employing an interresponse time schedule of reinforcement (IRT task) in which an animal is reinforced for pressing a lever once every N sec within a specified period ("limited hold"). If the animal presses too early or too late, the timer recycles. In these studies the animal was reinforced with a tiny squirt of apple juice for pressing the lever every 5 sec within a 2.50 sec limited hold. Exposures were 4 hours long and test periods were 3 hours long. In approximately 300 experiments, four 4-hour replications of each field exposure plus intermingled no-field tests were done for each of 5 monkeys. Evidence was discovered for a shift in the direction of shorter interresponse times in the presence of fields of a given frequency and voltage within a range from 1-56 V/m and 7 to 75 Hz.

Figure 1 shows the kind of IRT distributions that were observed for a single 4-hour exposure for a given field condition for one monkey and compares that distribution with a control, no-field test for the same monkey. Note the larger sample of responses in each distribution and the increasing separation of the distributions as voltage is increased from 1 to 56 V/m p-p.

Figure 2 summarizes the result of all of the experiments over all monkeys within the voltage range from 1 to 56 V/m p-p. The x-axis shows changes in

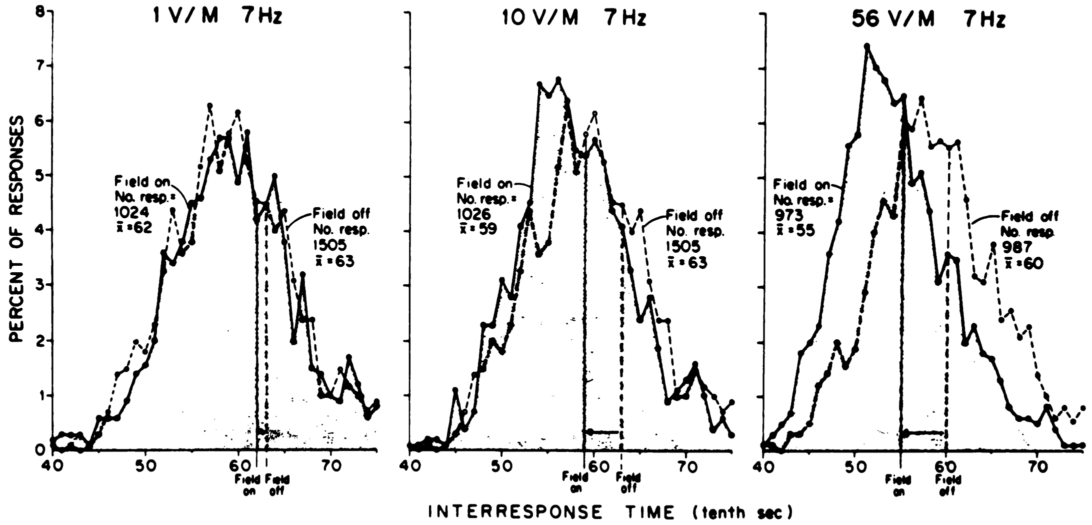

Figure 1. IRT (interresponse time) histograms are shown for a single subject (Mcaca Nemestrina) are shown for field (filled circles) and no-field, control, (open circles) sessions at 1, 10, and 56 V/M p-p with a 7 Hz modulation frequency. Each histogram was constructed from the data from a single experimental session. The vertical bars indicate the means of each distribution. Reproduced from (5).

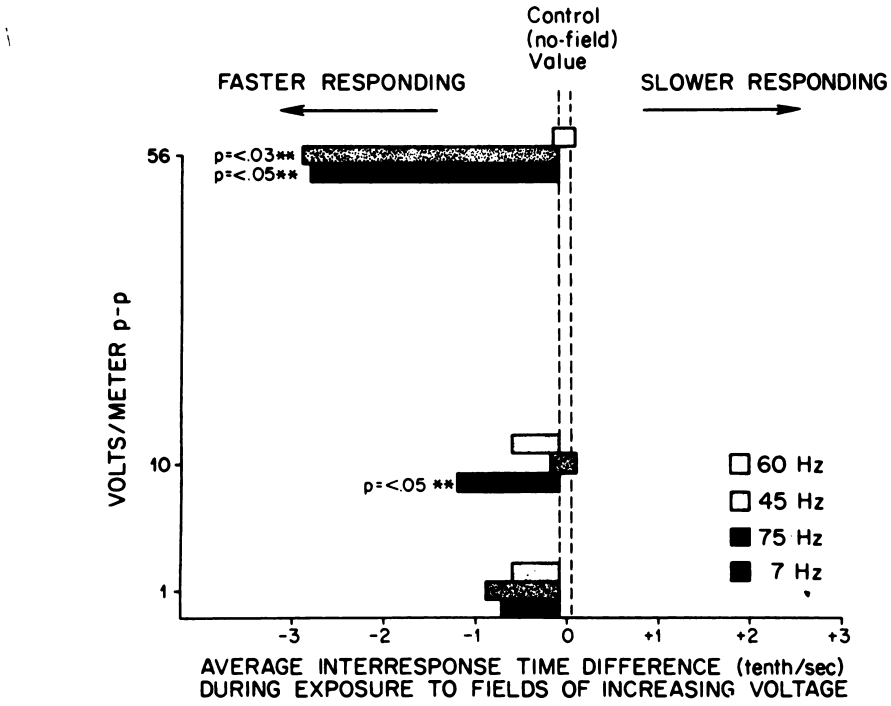

Figure 2. The average IRT difference between field and control sessions is plotted as a function of field strength in V/M. Reproduced from (5).

average interresponse times, the y-axis shows the three voltage levels tested (1, 10 & 56 V/m; the different bars represent frequencies tested). It may be seen that at 1 V/m, all differences are in the direction of shorter IRT's but none are statistically significant. At 10 V/m there is evidence for a threshold change for 7 Hz but not 45 Hz. This may represent the biological relevance of this frequency; it is within the range of hippocampal theta for the monkey. At 56 V/m IRT differences are much larger and significant for both 7 Hz and 75 Hz. Studies of EEG in two implanted monkeys in this series pointed towards a reduction of power in the range of 1-3 Hz and shift towards higher power in the middle EEG ranges of 5-16 Hz. Such data are compatible with heightened arousal and shorter IRT's.

Four major conclusions may be drawn from this study:

(1) *Frequency-specificity.* The evidence for a low threshold for 7 Hz is most interesting. Analagous frequency-specific changes in calcium efflux (6) in in vitro neo-natal chick brains have been observed in our laboratory.

(2) *Voltage.* The data suggest some degree of dose-dependency. Results at 100 V/m p-p were inconclusive and suggest either a voltage-window of the kind observed by Kalmijn (and in the calcium efflux studies) or a 24-hour carryover effect.

(3) *Duration of exposure.* Relatively long exposure durations appear to have contributed to the systematic array of results.

(4) *Behavioral assay.* *The question of external stimulus control.* The IRT task apparently was adequately sensitive and reliable. A comparison of our results with those of other negative primate studies revealed that in the negative studies, behavioral assays included more traditional tasks including reaction time tests, fixed interval tests, match-to-sample tests, etc., typically administered in 15 minute intervals. Animals were deprived of food or water and exposed to a variety of light and sound cues regulating the varius tasks. In the IRT task, the monkeys were isolated; they were not deprived during testing; there were no sound or light cues regulating their behavior. It might be said that the animals were forced, by the nature of the timing task, to pay attention to their own internal milieu.

A quick reminder - note that this is quite analogous to Keeton's interpretation of the pigeon homing data. This general finding might be paraphrased as follows: Behavioral effects, if any, of weak electric fields are *more likely to be observed in the absence of strong external stimulus control.*

A similar interpretation has been made in a number of studies in the area of behavioral toxicology. Figure 3 shows the results of a study (7) of the effects of amphetamine on a DRL (differential reinforcement of low rates) schedule of reinforcement. This schedule is simular to the IRT schedule, but lacks a limited hold. This schedule revealed a substantial effect of 1 mg/kg of amphetamine on both number of responses and number of reinforcements. When the experiment was modified so that a single external stimulus cue (a light) was added to the onset of correct interval, the effects of amphetamine were completely obliterated.

Similarly, data from Laties (8) is presented in figures 4 & 5. This study demonstrates that a pigeon worked on a FCN8 (fixed consecutive number) schedule of reinforcement showed extremely variable performance following administration of methyl mercury. However the addition of a light cue, indicating when the animal should shift to the reinforcement key, resulted in "normalizing" his behavior so that the effects of the methyl mercury could no longer be observed.

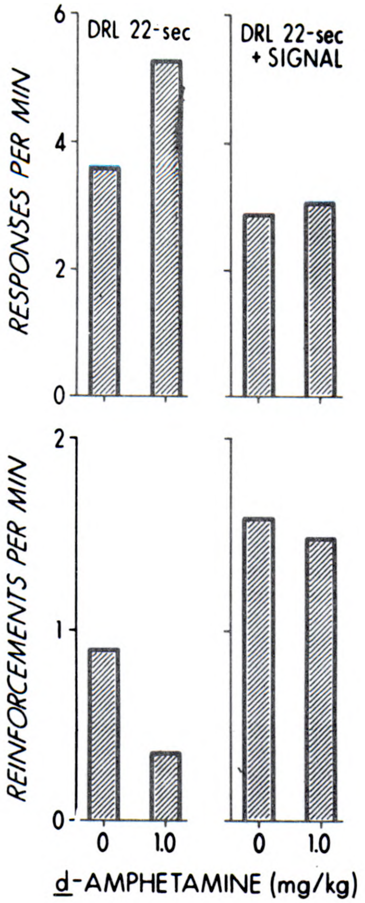

Figure 3. The effects of external stimulus control, in the form of a "behavioral prosthesis," on performance on a DRL schedule are illustrated. The effects of the administration of d-amphetamine are abolished when the external stimulus indicating reinforcement availability is introduced. Reproduced from (8).

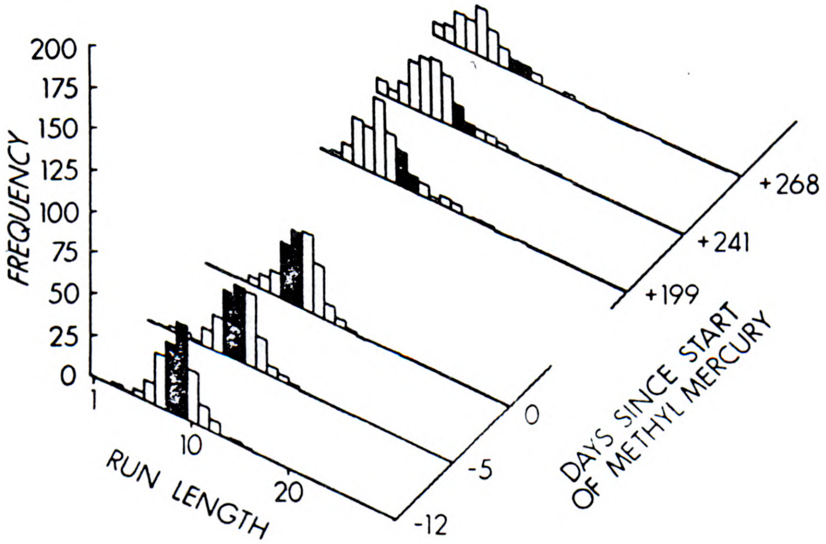

Figure 4. The effects of methyl mercury on pigeons working on an FCN 8 or 9 schedule. Reproduced from (8), data from (7).

The subsequent removal of the light revealed that the behavior once again appeared perturbed--and implied that the effects of the mercury were still present. Ogden Lindsley has aptly labelled the use of such cues a "behavioral prostheses" (8).

Finally, a similar example of the effect of external stimuli control was reported in a recent issue of the Journal of Comparative and Physiological Psychology by Braggio & Ellen (9). In studies of brain lesions in the septum, hippocampus, dorsomedial nuclei of the thalamus and dorsoventral thalamus they found that behavior on a DRL schedule is disrupted (over-responding occurs). The authors note that adding a light as a timing cue attenuated the symptom, and "... eliminates the appearance of any difference between operated and normal animals during the cued training period" (9)

Application of These Findings to Strategies for New Studies with VHF (450 MHz) Fields.

These conclusions, drawn from our own ELF work and the studies in behavioral toxicology, have markedly influenced the research strategies adapted for our new experiments with weak amplitude-modulated 450 MHz. Very briefly, these considerations have resulted in the development of a "three dimensional" programmatic approach to assay field effects on behavior.

(1) Experimental tests will be done across power levels with special attention given to the possibility of voltage "windows".

(2) Tests will be made across modulation frequencies with special attention being given to biologically relevant (i.e. within EEG range) frequencies.

(3) Tests will be made across increasingly complex time-based schedules of reinforcements.

A smaller, less expensive animal, the neonatal chick will be used in these studies. This will enable testing of relatively large numbers and will allow us to look for concomittance with the neurochemistry (especially calcium efflux) studies already in progress. In addition, it is proposed to contrast the chicks behavioral data with that of neonatal wild mallard ducklings to determine if special sensitivities exist in a migratory species.

MATERIALS AND METHODS

Neonatal chicks and ducklings will be used in a programmatic series of studies. A special activity monitor made of polyethylmethacrylate with a dielectric constant of 2.52 at 10^8 Hz has been fabricated. The Plexiglass chamber is 20 cm by 20 cm x 20 cm and uses three fiber optic light guides to monitor activity. A second, modified, chamber has been made which, along with the activity monitor includes a small Plexiglass reservoir arranged so that water can be made available for 2 sec and then drained.

The 450 MHz exposure chamber is a pyramidal horn 3 m long that tapers to a 1.5 m^2 exposure area lined with absorber. A maximum of 20 mW/cm^2 can be applied. It is possible to amplitude-modulate the 450 MHz signal at about 80-90 percent modulation at a frequency from 2 to 20 Hz. The chamber has been evaluated with a special probe developed by the Bureau of Radiological Health. Bassen (10) has described the chamber in more detail; he reports that the field is quite uniform in intensity and linearly polarized in the empty chamber. With a vertical E field, there is a narrow (50 cm) but high (150 cm) quiet zone, even with a monkey centered in this area in a plastic chair.

Proposed behavioral protocols and exposure parameters are described briefly below.

Note that in general, modulation frequencies at 3 Hz and 16 Hz will be tested. This reflects the fact that (1) EEG recordings from neonatal chicks indicate that 3 Hz is associated with somnolence and 16 Hz is associated with activity and (2) calcium-efflux experiments in our laboratory have indicated that there is a "tuning curve" over frequencies with maximum efflux at 16 Hz (11).

Exposure durations in these studies are quite short. Earlier studies in our laboratory at 147 MHz indicated weak fields affected EEGs in chronic cats after only 30 sec. Thus it is expected that, with sensitive behavioral assays, 20 min may be long enough.

(1) Simple Activity Tests. Field parameters: 1-5 mW/cm^2, c.w, 3 Hz, 16 Hz, modulation frequency, 70 percent modulation, Exposure = 20 min. Neonatal chicks and ducklings will be tested in an off-on-off paradigm in which the field is present for 20 minutes in the middle of an hour long test. Appropriate control tests performed in the same chamber, with the field off, will also be 1 hour long. Activity will be monitored. Special computer programs have been devised so that the tape recorded data can be described in terms of number of movements, time between movements, cumulative probability, etc.

(2) Fixed Time (F.T.) Schedule. Field parameters: 1-5 mW/cm^2, c.w. 3 Hz, 16 Hz modulation frequencies, 70 percent modulation. Exposure = 20 min. In the FT schedule, a reinforcer is delivered every N seconds independent of the animal's behavior, this has the effect of regularizing and increasing the amount of activity produced by an organism. It is a schedule that has been studied by Staddon & Simmelhag (12) and Killeen (13) in rats, pigeons and chickens and has been shown to be sensitive to drugs. Typically behavior under control by this schedule tends to show a peak in the middle of the FT interval.

In the present study the activity of chicks and ducklings will be assayed on an FT 30 sec schedule with water as a reinforcer (14). The off-on-off paradigm described earlier will be used and random 1 hour intra-individual no-field control tests will be made. Sixteen 12 day old x-link white leghorn chicks that are 23 hours water deprived will be tested. The first study in this series has been started. The power level was set at 5 mW/cm^2. Six 1 hour training sessions at the same time every day preceded a counter balanced schedule of two 1 hour, off-on-off hour tests at 3 Hz and 16 Hz and 4 hour control tests for each chick. Tests of unmodulated fields will follow.

Data will be analyzed on a PDP-12 computer for gross amount of activity, time between responses, time since last reinforcement, cumulative probability of responding since last reinforcement, etc.

(3) IRT Schedules. Field Parameters: same as for FT. This schedule has been described earlier, in these studies it will be used with water-deprived neonatal chicks with the interval set at 30 sec. to correspond with the FT schedule. A similar off-on-off exposure paradigm with 20 min of field exposure will be used.

(4) IRT Schedule with Cue. A light cue will be added to the IRT schedule. This will test the effect of external stimulus control in abolishing possible field effects.

(5) Discrimination tests, concurrent chain tests, and more elaborate behavioral tests will be assayed depending upon the results of the earlier, simpler tests.

RESULTS

A partial set of neonatal chicks (8 of 16) have been tested in the FT 30 sec. schedule described above with power set at 5 mW/cm^2. It appears that their behavior is regularized as the schedule predicts. A sample distribution of one chick's activity during the 30-sec interval between reinforcements, averaged over a 1 hour control test, is given below. (Fig. 6) Note the peaking of activity midway between reinforcements.

Preliminary graphs of the data suggested that gross amount of activity is not affected by either the 3 Hz or 16 Hz amplitude-modulated 450 MHz field.

Figure 7 shows the available data averaged over animals and equated for initial levels. All animals show an increased inactivity during the second 20 min. epoch (the field exposure epoch) regardless of whether they are being exposed to a field or not. Similarly they show a decline in activity in the third no-field epoch regardless of whether or not a field exposure preceded it. Statistical differences between conditions were not observed. Certainly the pattern of activity is similar for all three conditions.

When a slightly more sophisticated measure of behavior is used, however, differences begin to appear. Thus figure 8 shows a graph of the median latency of responses since the last reinforcer.

As one would expect the three conditions look very similar for epoch 1, a no-field period of 20 min. However, both the 3 Hz condition and the 16 Hz condition appear to be associated with markedly reduced latencies in epoch 2, the 20-min period during field exposure. Nonparametric statistical tests (sign tests) of the differences between median latencies for epoch 1 (no-field) and epoch 2 (field) were significant at the 0.03 level for both the 3 Hz and 16 Hz condition. (See Table 1.) The shift is 1.2 sec for the 3 Hz field and 1.1 sec for the16 Hz field. Similar tests in the same 8 chicks for the hour long control tests show that latencies are not different from epoch 1 to epoch 2.

Table 1. Median latency of activity since last reinforcement of an FT 30 sec. Schedule N=8. matched pairs sign test

	Epoch 1 (control)	Epoch 2 (field)
3 Hz mod.	15.4 sec	14.2 sec P=.03
16 Hz mod.	15.8 sec	14.7 sec P=.03
	Epoch 1 (no-field)	Epoch 2 (no-field)
1 hr. no-field	15.4 sec	15.2 sec P=.636 not sig.

Data from epoch 3 (the post-exposure period of 20 min), which might indicate the presence of carry-over effects, are inclusive at this time.

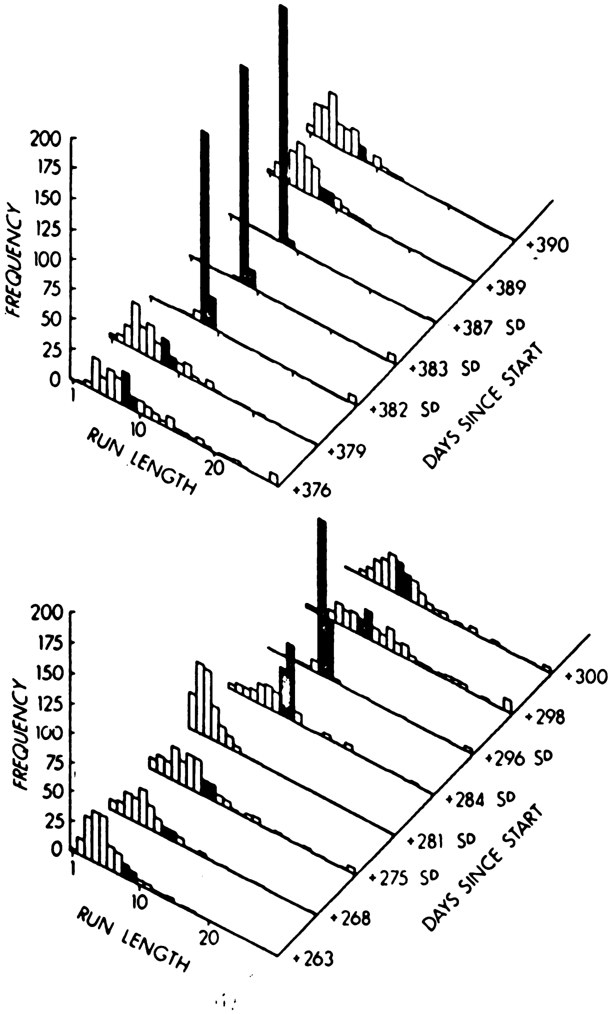

Figure 5. The effects of an external discriminative stimulus (S^D) on performance on an FCN 8 or 9 schedule following administration of methyl mercury. In this extension of figure 4, it can be seen that the introduction of an external discriminative stimulus leads to the abolition of the measured effects of the methyl mercury and the removal of the external discriminative stimulus leads to their reappearance. Reproduced from (8).

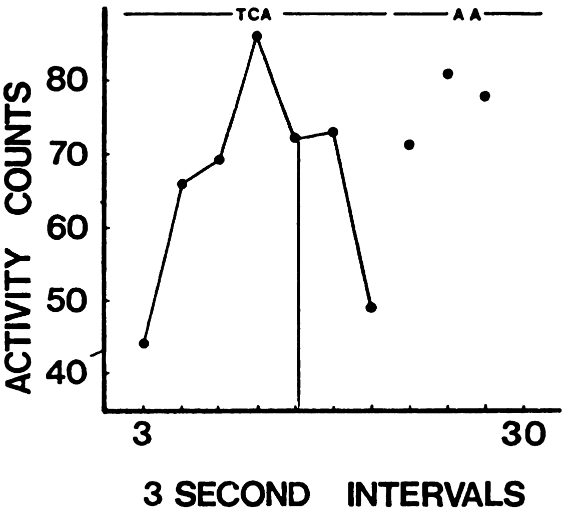

Figure 6. The frequency histogram of activity of differing latency since the last reinforcement on an FT 30-sec schedule of reinforcement is shown for a chick during a single 1h control session. The points connected by the line show the characteristic form of temporally controlled activity (TCA) on the FT schedule: activity is minimal immediately following a delivery of the reinforcement, rises to a peak near the midpoint of the interreinforcement interval, and then drops off as the time of the next reinforcement approaches. The unconnected points indicate approach activity (AA): as the time of the reinforcement approaches, the subject crosses the test chamber to arrive at the vicinity of the water cup. Approach activity is observed on the FT schedule when the size of the subject is small with respect to the size of the test chamber. The vertical bar indicates the median of the entire latency distribution.

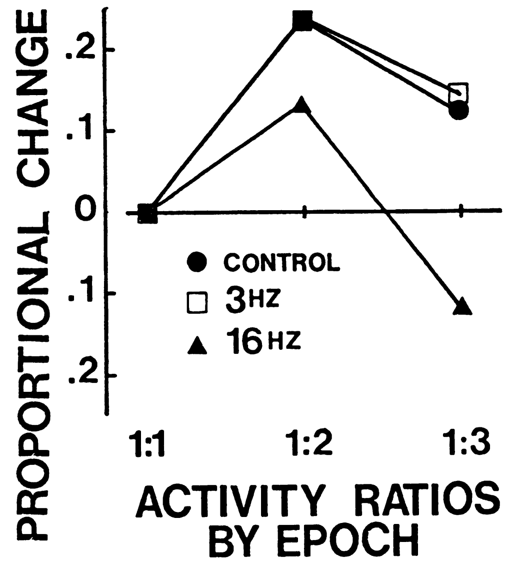

Figure 7. The amount of activity on an FT 30-sec schedule in each of the three 20-min epochs comprising the 1h session is shown normalized with respect to the amount of activity occurring in the first epoch. The activity amounts are the means for 8 chicks and are drawn from control, 3 Hz and 16 Hz sessions. Field exposures were determined randomly; in those sessions for which a field was scheduled, it was presented during the second (middle) epoch. No statistically significant differences were found between the amounts of activity in the various epochs as a function of the presence of the 450 MHz field. Field strength was 5 mW/cm^2 and modulation depth ∿ 70 percent.

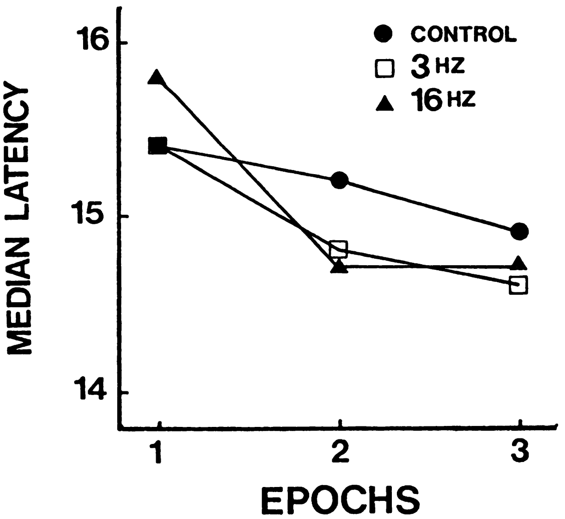

Figure 8. The median latency of response on an FT 30-sec schedule for 8 chicks is shown as a function of epoch with field condition (control, 3 Hz or 16 Hz) as the parameter. The field condition was determined randomly; in those sessions for which a field exposure was scheduled, it was presented during the second 20-min epoch of the 1h session. Sign tests of the differences of the median latencies for epoch 1 and for epoch 2 were significant beyond the 0.03 level for the 3 Hz and 16 Hz conditions, but were not significant for the control condition. The data contrasting epoch 1 to epoch 3 are inconclusive at this time. Field strength was 5 mW/cm^2 and modulation depth ∿ 70 percent.

Further testing is being done to confirm these preliminary observations. It seems likely that the differences in latency will persist when more animals are tested. These data suggest that the time dependent activity controlled by an FT schedule is systematically shifted in the direction of shorter latencies in the presence of the low frequency, amplitude-modulated 450 MHz field.

These results suggest further that time dependent behavior, measured in terms of latency rather than in terms of gross activity can be a sensitive and reliable indicator of weak field effects. In this instance, the 450 MHz field has an incident power of only 5 mW/cm^2 and exposure time is only 20 minutes.

Finally, it may be noted that heating, with an external heat source, generally produces decreased activity in chicks (15). In these experiments, activity per se was not affected. The decrease in latency observed also seems to be incompatible with an explanation in terms of mediating micro-changes in temperature. Whatever small internal changes in temperature may have been concommmitant with the field exposure are not likely to have produced the kind of behavioral perturbation observed.

DISCUSSION

Behavioral studies of the effects of weak electric fields from ELF to microwave range present a contradictory array of results. In part this reflects great discrepancies in the physical parameters of the field employed. In particular, (1) little attention has been paid to the biological relevance of the frequency (either c.w. or modulation frequency), (2) the question of possible voltage or power windows and (3) the duration of exposure. Very few studies have systematically varied one or the other of these parameters in a way which might reveal dose dependencies. The apparent arbitrary choice of parameters may reflect, in part, an implicit concern with "hazard", at one extreme or with "thresholds" at the other. In the case of microwaves, especially, many behavioral assays have been directed at discovering the power level at which the animal is almost totally incapacitated. Such findings have obvious practical implications but do little to elucidate the question of behavioral thresholds. A direct corollary of this may be that only threshold studies -- and not hazard studies -- are likely to supply clues about possible subtle central nervous system mediators.

A brief review of work in this laboratory in ELF studies with primates, taken in conjunction with recent studies in behavioral toxicology and some preliminary data from amplitude-modulated VHF studies, all suggest that the role of external stimulus control is important in determining the adequacy of a behavioral assay -- especially if one is concerned with threshold detection. Behavioral experiments with many external stimuli controlling the behavior in question may be intrinsically insensitive to the presence of weak, background, electric fields.

Behavioral tests without these constraints, which involve time-dependent schedules, appear to offer special promise.

CONCLUSION

The apparently contradictory results of behavioral studies of weak electric fields may be a function of 1) precise physical parameters of the field and 2) the use of behavioral assays that differ markedly in their degree of external stimulus control. ELF primate studies and preliminary data form studies of neonatal chicks exposed to weak amplitude-modulated VHF fields suggest that random activity per se is too variable to be a reliable measure of weak field effects. At the other extreme, behavioral tests which involve an excessive degree of external stimulus control may mask weak field effects. A programmatic study of relatively simple to relatively complex time-based schedules of reinforcement has been undertaken to further elucidate this issue.

ACKNOWLEDGMENT

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OPEN DISCUSSION

DR. OSEPCHUK: Can you describe the physical exposure system?

DR. MEDICI: I think the 450 MHz exposure facility is probably going to be described by Mr. Bassen later on. Basically, though, it is a pyramidal horn, 3 meters long; it tapers to a 1.5 meters square exposure area that is lined with absorber; and the power that we can deliver is up to 20 milliwatts per square centimeter. The field was measured by Mr. Bassen and was described as quite uniform in intensity and linearly polarized in the empty chamber. We also did measurements with animals in the chamber.

DR. OSEPCHUK: What kind of chamber was it?

DR. MEDICI: There were two separate chambers. The ELF studies were done in a screened room, and the field was administered to two large metal plates on either side of the animal.

DR. OSEPCHUK: Do you have any idea of the distance between the plates?

DR. MEDICI: The plates were 1 meter square and 0.5 m apart.

DR. JUSTESEN: This has all been published in a May 1976 issue of Nature.

MICROWAVE HYPERTHERMIA AND VISUALLY EVOKED POTENTIALS: PRELIMINARY OBSERVATIONS OF GUINEA PIGS

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ABSTRACT

Latencies of an early component (N_1) of the Visually Evoked electrocortical Response (VER) of guinea pigs were observed as a function of body temperature as elevated by brief exposures to intense 2450-MHz microwave radiations. A curvilinear relation obtained in which the N_1 latency decreased as temperature fell from 41° to 39.5° C, then increased as body temperature continued to fall to 38° C. The temperature for optimal rates of neural processing of sensory information may therefore be 1 to 2° C above that of the resting norm. Data from other studies indicate that elevations of body temperature to 39-40° C occur to novel stimuli and other sources of emotional provocation. The inverted "U"-function, if a valid reflection of the normal relation between body temperature and neural activity, can be taken as evidence for an adaptive role of the "psychogenic fever" that attends behavioral hyperarousal.

INTRODUCTION

As the temperature of nerve fibers is elevated toward the biological norm, there is a reliable increase in conduction velocity both in the in vitro and in the in vivo preparation (1, 2, 3, 4). The acceleration of biological activity that accompanies moderate heating of the central nervous system must have a limit at some hyperthermal point since the crude electroencephalogram (EEG) of the mammal disappears completely during induction of intense, nonmortal fevers (5). Currently unknown is the form of the function that relates supranormal temperatures of brain or lower body to rates of nervous activity of intact, multineuronal populations of cells. We are atempting to shed light on this function through microwave thermalization of the guinea pig and near-concurrent observations of latencies of early components of the VER. The rationale for selecting the evoked response as an endpoint is rooted in its invariant character in the intact animal and in its sensitivity to individual differences and to cerebral insult (see Fig. 1) (6). The rationale for selecting the guinea pig as an experimental subject is related to the requirement for a stable VER as evoked by a single photic stimulus, i.e., the VER is typically the computer averaged resultant of a large number of photic stimulations. Since the aim is to raise an animal's temperature during a short period of microwave radiation and then to observe VERs during return of body temperature to the preradiation level, any thermally dependent changes of electrocortical activity would be confounded during a protracted series of stimulations because of the fall of temperature across time. At least for latencies of early components of the VER, the guinea pig's single evoked response has ostensibly greater invariance than does that of the rat or cat (Fig. 2).

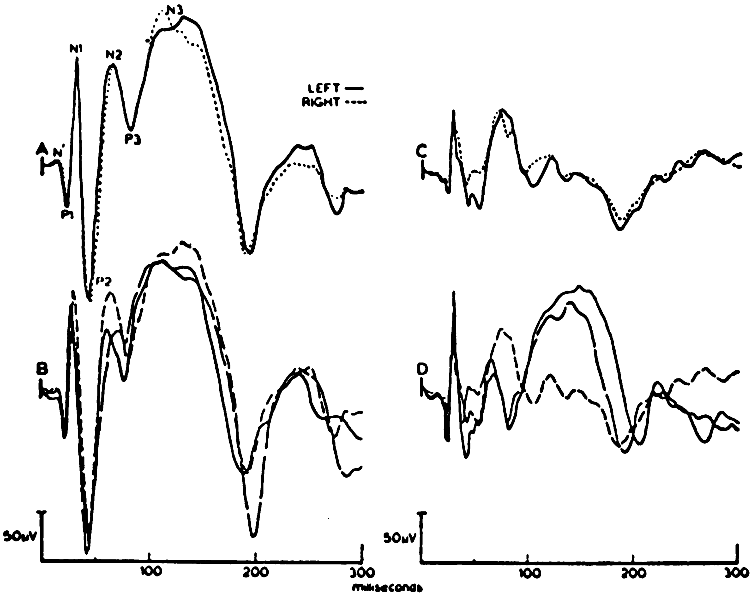

Figure 1. Signal averaged, visually-evoked electrocortical responses (VER's) of guinea pigs under binocular stimulation. Top tracings: the solid line depicts the VER as recorded from the left hemisphere; the dashed line, from the right hemisphere. Bottom tracings: left-hemispheric responses as measured three times at separations of several weeks. Left side tracings are from an albino; right side, from a visually pigmented guinea pig. The alpha-numerical symbols for the various peaks of components indicate polarity (N= negative, P = positive) and order (1 = 1st, 2 = 2nd, etc.) of occurrence of early components of the VER, i.e., those occurring within 150 msec. of photic stimulation (from Creel, 1969).

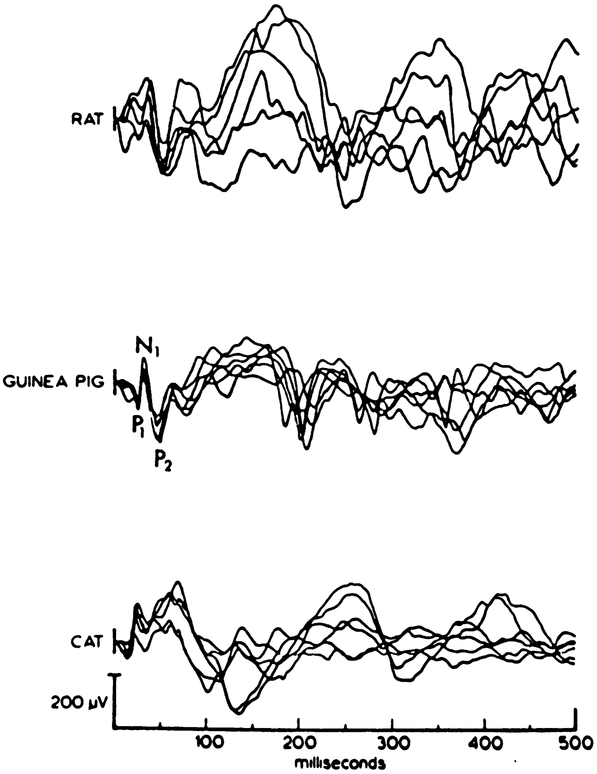

Figure 2. The tracings are VERs, each one to a single photic stimulus, from a rat, from a guinea pig, and from a cat. The six VERs of each species are to the 1st, 10th, 20th, 30th, 40th, and 50th photic stimulus in a series of 50 consecutive stimulations. Note the greater temporal consistency of early components, particularly N_1, of the guinea pig's VER as opposed to that of the rat and of the cat (from Creel, 1969).

MATERIALS AND METHODS

Two guinea pigs of the Hartley strain, both mature males, were stereotaxically fitted under anesthesia with chronic recording electrodes that were located, one to the left and one to the right, 6-mm lateral to the sagittal suture and 2-mm anterior to the lambdal point. A third, reference electrode was placed at the midline 12-mm anterior to the bregmal suture in the frontal bone. Each of the three electrodes was a small, stainless steel screw that penetrated the calvarium to contact the dura mater. The area of each of the screws that contacted the dura is 1.5^2 mm. The heads of the screws and the insulated copper wire to which each screw was connected were covered on the external surface of the calvarium with dental acrylic. The distal ends of the copper wires made contact with connector pins in a small amphenol plug that was secured to the calvarium with acrylic. The placement of the screw-electrodes was accomplished with the stereotaxic instrument of Justesen, et al. (8)

After surgery, the animals were habituated 10-15 min. daily for a period of two weeks to restraint in a canvas hammock. Thereafter, an animal was placed in the hammock with its head approximately 50 cm away from the lamp of a Grass Model PS-1 photic stimulator. The stimulator was adjusted to deliver a flash of light, of 25 µsec. duration, that approximated a peak illuminance of 1500 luces at an animal's eyes.

During elicitation of responses, a flexible set of conductive leads was attached to an animal's Amphenol plug by way of patching EEG signals into the electrode board of a Grass Model 6B/8-2 polygraph. The polygraph provided an ink-written record of the crude EEG and also served to filter and balance an amplified signal to an averaging computer (Nicolet Model 1052 LSH) that was used to store a single VER for production of a grpahic record by a Hewlett-Packard Model 7005-B X-Y plotter (see Fig. 3 for a block-diagram of the experimental set-up).

Radiation by 2450-MHz energy was carried out in the multi-mode cavity of Justesen, et al. (9). Just prior to radiation, an animal was gently but firmly wrapped in a folded hand-towel with its head protruding and its posterior availing of insertion of an insulted thermistor into the rectum. The thermistor's leads passed through a small hole in the viewing grille of the multi-mode cavity to a United Systems Model 1501 electronic thermometer. Microwave energy that was modulated as a half-wave sinusoid at 60 Hz was applied at the rate of 30 mW/g until the rectal temperature reached 42° C. After the animal reached this temperature, it was removed from the cavity along with the thermometer-probe and was transported a few meters to an adjacent, electrically shielded chamber for near immediate commencement of observations of VERs. As rectal temperature fell by half-degree decrements the photic stimulator and the signal-storage computer were manually triggered and an X-Y plot was made that displayed a VER over 500 ms from time of each photic stimulus.

RESULTS

Each of the guinea pigs underwent a minimum of 10 radiation treatments with accompanying observations of the VER. Each treatment was separated by a minimum of 23 hours. When latency of the first prominent negative (N_1) component as rounded to the nearest millisecond was plotted against body temperature at time of production of a VER, a clear-cut curvilinear function was observed (Fig. 4). The inverted "U"-shaped functions we observed have a minimum latency near 39.5° C, the latencies being longer at lower and higher temperatures. Other early components (P_1, P_2 and N_2) of the VER exhibited commensurate changes of latency in relation to temperature. While the general form of the function persisted

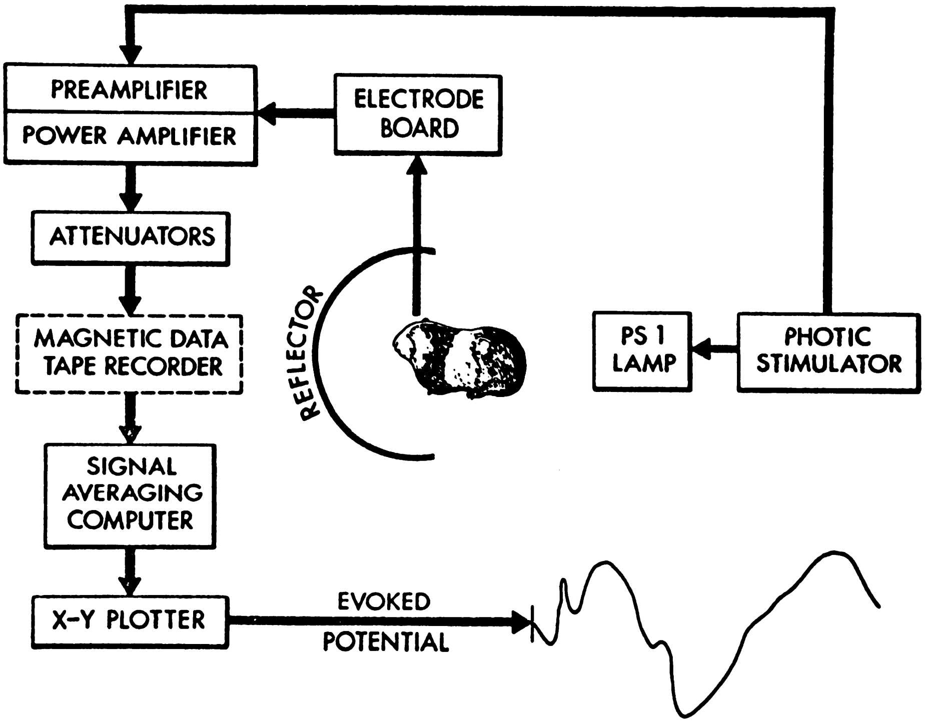
PREAMPLIFIER
POWER AMPLIFIER
ELECTRODE BOARD
ATTENUATORS
MAGNETIC DATA TAPE RECORDER
REFLECTOR
PS 1 LAMP
PHOTIC STIMULATOR
SIGNAL AVERAGING COMPUTER
X-Y PLOTTER
EVOKED
POTENTIAL

Figure 3. Block diagram of apparatus that was used to produce and record VERs from guinea pigs. While a large parabolic reflector is often used to present a constant-intensity Ganzfeld to an animal-subject in VER-studies, the guinea pigs of the present study were stimulated directly by the photic lamp. The magnetic tape recorder was not used in generation of the data that are shown in figure 4.

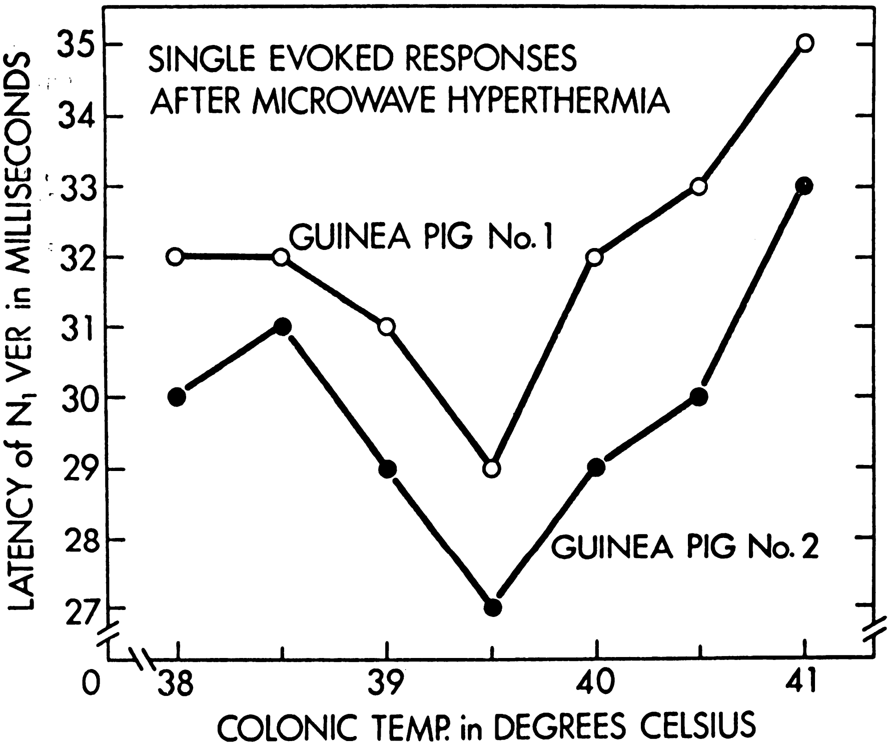

Figure 4. Plots of latency of the N_1-component of the VER as a function of rectal temperature of two guinea pigs. The data-points for each animal were generated during a single experimental session after heating of an animal to 42° C (rectal temperature) in a microwave field. Highly similar inverted "U"-shaped functions were generated during several sessions that were conducted daily across a total of 10 days.

throughout the series of treatments and observations, the latencies were found to vary somewhat, especially with changes of ambient illumination. The shortest latencies occurred when microwave treatments and observations of the VER were carried out in the near-darkness.

DISCUSSION

The finding of a minimum latency of the guinea pig's VER near 39.5° C accords with recent observations by Putthoff, et al. (10), who found that this species normally has markedly high body temperatures near 39.5° C when subjected to handling by human beings in a novel environment. Previous investigators have shown that behavioral and electrophysiological arousal, which are prompted by novel stimuli, are associated with a rise of body temperature (11, 12); it is reasonable to conjecture that the quickening of information-processing and of reflexive activity that accompany a rise of body temperature has survival value.

One does not make a case for temperature-optimization of survival on the basis of a pilot study of two guinea pigs; rather, this is the working hypothesis of a large-scale study in which we are attempting to answer several questions:

1. What role in production of the VER does the presence of chronically implanted electrodes play in an animal that is irradiated by intense levels of microwave energy? More speacifically, will shielding of the head or post-irradiation insertion of recording electrodes or heating of animals by conventional means yield the same inverted "U"-function?
2. Will behavioral measures of thresholds of perception of microwave energy be altered by the presence of chronically implanted electrodes:
3. Will post-mortem histological analysis of chronically implanted animals reveal signs of tissue-injury at loci where electrodes have contacted the brain?
4. What is the concordance between post-radiation temperatures as measured by chronically indwelling and by acutely fitted sensors in the lower body and in the brain?

CONCLUSIONS

The latency of the N_1 component of the VER, which probably reflects arrival at the cortex of a neural volley along the retino-geniculo-striate pathway (13) was found to decrease and then to increase as body temperatures of microwave-thermalized guinea pigs fell from 41 to 38° C. Barring artifact, the inverted "U"-function may be taken as evidence of optimization of rates of neural activity and information-processing as the guinea pig's body temperature is normally elevated to temperatures near 39.5° C during behavioral hyperarousal.

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OPEN DISCUSSION

DR. CARPENTER: Have you tried the pigmented guinea pig in comparison to the albino?

DR. JUSTESEN: Yes. While the data of figure 4 are based on two albino animals of the Hartley strain, we have recently obtained data on pigmented guinea pigs. The amplitudes of the VER-components are generally higher in the albino but the latency of the early, N_1 component tends to be shorter in the pigmented animal. Yours is a good question in the light of ever expanding data that there are profound neurophysiological differences between albino and pigmented mammals.

DR. CARPENTER: You mentioned the non-pigmented eye had less scattering than the pigmented one.

DR. JUSTESEN: In the dark-sensitized animal, amplitudes of early VER-components are considerably higher in the albino to a given photic stimulus. I interpret this difference to result from general absence of pigment in the ocular apparatus; that is, I believe in the albino animal that a photon of light has a higher probability of being captured by a receptor after penetrating cornea, iris, ocular humor, and the layers of bipolar and amacrine cells--all of which are interposed between the periphery and the receptor.

DR. CARPENTER: The pigment is behind the external segments, and around the external segments are the rods so that it does not interfere with the path of light stimulus.

DR. JUSTESEN: You are talking about the choroid layer that does lie distal to the receptors. My point is that more photons reach the receptors in the albino. How else to explain the higher amplitude of the VER?

DR. CARPENTER: You presented data on cats in one of your slides (Fig. 2). Now a cat has a tapetum lucidum--a reflecting light behind the retina, and that is a scattering device. Any animal from which you get a light reflex at night has some sort of a scattering device. What that does is to reinforce the sensitivity of the eye. I would expect that the albino would have much more scattering, because the light goes through to the visual cells and then strikes the pigment epithelium which has very little, or some, pigment in the albino, and then it strikes the choroid with a layer which could be reflective. However, I do not think that there is any special tapetum in the rodents.

DR. JUSTESEN: No, there is not.

DR. CARPENTER: We find the tapetum only in the carnivores and the ungulates. Therefore, I would expect that there is more scattering with the albino than with the pigmented; because the pigment absorbs light that is not affecting the rod, and it absorbs light that has affected the rod. Thus, it cannot bounce back and hit it a second time.

DR. JUSTENSEN: There is a sizeable comparative literature on the subject of the visual anatomy and physiology of the albinic mammal, much of it generated by Donnell J. Creel of the Salt Lake City Veterans Administration Hospital. He found that albinos after maintenance under moderate to high levels of ambient illumination show a poorer VER, presumably from greater "bleaching" of visual pigment. After dark-sensitiation, higher amplitudes are observed in the albino. I ascribe these differences to the greater likelihood that more light will traverse the poorly pigmented tissues of the iris in the albinic animal with the consequence that more light reaches the receptors.

DR. SESSION: You seem to be describing a very elegant demonstration of an arousal phenomenon here, would you agree?

DR. JUSTESEN: That is precisely what I think it is, but credit Mr. George Sacher of the Argonne National Laboratories with the insight for a most intriguing theory: the nervous system of the mammal has evolved as a metabolic modulator. Under resting conditions--in the absence of threatening or alerting stimuli--the mammal conserves metabolic energy, is "idling" as it were. Stimuli that arouse the nervous system produce metabolic activation. The body is literally "fixed up" for fight or flight.

DR. SESSION: I can think of several interesting implications of your work unrelated to microwaves but with respect to microwave energy, would you hypothesize that the guinea pig has his own mechanism for getting its temperature up to 39.5° C as you have demonstrated here? Would you, further hypothesize that if you could keep the animal at that temperature by the application of microwave energy that you would produce behavioral changes in that animal: Further, would you hypothesize that animals that were allowed to arouse themselves, would then cool down? I am thinking of applications here; I am trying to pick out what you have in mind for application.

DR.JUSTESEN: To respond to your questions in order, first, the guinea pig demonstrably has its own mechanism for raising its temperature to 39.5° C or therabouts. Indeed, unless the animals are repeatedly "gentled," acclimated to handling in the laboratory, it is difficult to obtain body temperatures that are less than 39° C. In other words, being accosted--being picked up--by a human being is a behaviorally and metabolically arousing stimulus for the guinea pig. Dr. Henry Ho has obtained data that respond to your second question: Mice maintained in a febrile state by continuous-application of microwave energy in a waveguide show a moderate but reliable reduction of metabolic rate. This apparent conservation of endogenous energy was also predicted by George Sacher. I have already responded in part to your last question. Even as presentation of a novel stimulus--handling--arouses the guinea pig, as evidenced by elevation of body temperature, removal of the stimulus, as evidenced by thermal telemetry after cessation of handling, is followed by a fall of body temperature. This is not to say that the guinea pig controls or "wills" the rise and fall, only, in my opinion, that we are seeing adaptive reflexes that are "wired" into the mammalian nervous system.

Before departing from our demonstration of an apparent thermal optimization of nervous activity, I want to ventilate a concern for a possible artifact. The data of figure 4 are based exclusively on descending series, that is, VERs were measured while body temperatures fell from a peak value near 42.5° C. It is possible in conducting the descending series that the prior, rapid incrementing of body temperatures to 42.5° C by microwave radiation may alter photochemical or nervous activity in such a way that VERs measured thereafter at lower temperature are contaminated. It is an axiom of psychophisical methodology that a descending series must be complimented by a ascending series. We are currently performing the latter by raising body temperature by radiation in small increments, by an additional 0.5 to 1.0° C on each succeeding day, but have yet to obtain data beyond 41° C. The inverted-"U" function appears to hold although more data points are needed to make a complete case.

Finally, I'd like to mention a bit of fallout from recent studies where we have measured colonic and brain temperatures simultaneously, both before and after microwave radiation of the guinea pig. We were surprised to find that pre-exposure, "resting" temperature of the brain is as much as 2° C below the colonic temperature. The difference narrows as temperature is elevated by microwave irradiation and there are indications that brain temperature eventually exceeds that of the colon, although the presence of small metal electrodes that contact the brain may be a contributing factor.

INTERACTION BETWEEN MICROWAVE AND NEUROACTIVE COMPOUNDS

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ABSTRACT

Microwave radiation can produce an alteration in the behavior of an irradiated organism. It is commonly asserted that such behavioral effects are the result of a direct action by microwaves on some unspecified aspect of the central nervous system. However, the data to support such an assertion are weak. A study is reported which attempts to elucidate a neurotransmitter-linked mechanism of microwave action.

INTRODUCTION

The existence of behavioral changes induced by microwave radiation cannot be doubted. Unfortunately, the conditions under which such changes occur and the mechanism(s) responsible are essentially unknown. Although it is often assumed that observed behavioral changes are due to an interaction of radiation and CNS tissue, this assertion has not been convincingly demonstrated for most behavioral effects. Several lines of evidence suggest that such an interaction with the CNS is not a necessary condition for behavioral change. For example, the most reliable neuro-behavioral effect of microwave radiation, the microwave hearing phenomenon, can be adequately explained by invoking a peripahereal mechanism (1,2,3). A second highly reliable behavioral phenomenon, microwave avoidance (4,5,6) also can be readily explained without invoking a site of action in the CNS. In fact, microwave avoidance and other behavioral phenomena may be more plausibly explained without invoking a central mechanism (7).

A behavioral effect which appears after cessation of irradiation is more difficult to explain without invoking a central mechanism (8,9). Nonetheless, direct evidence for the existence of a mechanism, operative in vivo and necessary or sufficient to produce a behavioral change, is at best scanty.

At the cellular level, no morphological changes have been reported in the following irradiation at levels less than about 10 mW/cm^2 (10), although behavioral effects have been observed at lower power levels. Similarly, significant changes in neurotransmitter concentrations have not been reported at power densities less than 10 mW/cm^2, although high power densities have profound effects (11).

Adey and his associates (12,13) have drawn upon classic theory of neural membrane function to propose an elegant hypothetical mechanism to explain how microwaves might interact with neural tissue by inducing conformational changes in calcium binding sites on dendritic membranes. While the hypothesis permits accurate predictions regarding the behavior of in vitro preparations in microwave fields, much research remains to be done before it can carry the burden of explaining alterations in the overt behavior of an intact organism.

Meanwhile, we have adapted an indirect approach to the search for a microwave sensitive system within the CNS. In an early study, we (**14**) observed that a performance deficit could be induced in rhesus monkeys by irradiating the entire head with fairly high levels of microwave radiation (integral dose rate ∽ 15W). The most striking aspect of these data was the time course of the effect. The subjects were irradiated daily for 5 days and the performance deficit typically became observable on the 3rd day, increasing with each passing day. Furthermore, the effect was at least partially reversible. This suggested to us a possible biochemical basis for the behavioral suppression.

Our next step in investigating this hypothesis was to study the effects of combined doses of microwave radiation and serotonin-depleting drugs. This study was reported at the 1976 meeting of the Southeastern Psychological Association Meeting held in New Orleans, Louisiana. Response rates in a four-choice reaction time task were measured. Each monkey was trained to choose the illuminated one of four locations. A food pellet was delivered to the monkey upon completion of 100 correct choices; a short tone signalled each correct choice. Errors resulted in a time-out in the dark, thus delaying the onset of the next trial. Baseline rates were obtained for at least 5 days before each insult. Subthreshold levels of PCPA (p-chlorophenylalanine)or fenfluramine were administered and microwave energy at 383 megahertz was delivered to the head of the monkey in "doses" ranging from 10.0 watts to 25.0 watts. Irradiation of the monkeys combined with fenfluramine administration resulted in a decrease in response rates greater than that obtained by either microwave or fenfluramine alone. The combination of PCPA and irradiation resulted in decreases in response rates less than those due to microwave and fenfluramine.

In the present experiment we extended our research to another behavioral task and another microwave frequency.

MATERIALS AND METHODS

Subjects were four male rhesus monkeys, 7-9 kg in weight. The subjects had been exposed to a variety of drug and microwave radiation regimes while performing a match-to-sample task prior to the onset of the present experiment.

The apparatus contained three acrylic plastic stimulus panels, arranged in a horizontal row. Each panel could be lighted either red or green. Below each stimulus panel was a response lever. For each trial the center panel was lighted, red or green, a single response on the center lever turned off the center panel and simultaneously lighted the side panels, one red and one green. Which side panel was red and which green varied randomly from trial to trial. Correct matches on the side levers were reinforced on the average of once a minute (a variable interval 1 minute schedule). Incorrect responses produced a 10-second panel blackout during which responses produced no effect.

Each subject was exposed to CW 2450 MHz microwave radiation at each of six levels (integral dose rates: 1, 3, 5, 10, 15 W) in the irradiation apparatus described below. Each subject was also injected i.m. with various doses of fenfluramine dissolved in a constant volume of one cc of physiological saline. For each subject, the highest dose of fenfluramine that did not produce a change from non-drug baseline behavior was administered in combination with an integral microwave dose rate of 15 W. The irradiations were performed in the experimental chamber during selected sessions. The subjects were seated in a restraining chair, which was raised to a position in which the top of the subject's head fitted snugly into a circular hole cut in the bottom of a section of a rectangular waveguide. Power absorbed by the monkey's head was estimated by subtracting the reflected power from the power fed into the waveguide (15).

RESULTS

For each session, eight behavioral measures were calculated: percent correct red and green matches, percent responses on the red choice key, percent responses on the right-side key and response rates of correct and incorrect red and green key responses.

A dose of fenfluramine was found for each subject which resulted in a decrease in response accuracy and in a decrease in response rates. The minimum effective dose ranged from 0.2 mg/kg to 4.0 mg/kg.

Integral dose rates of 1-15 W of microwave radiation produced no change in the behavior of any subject. Limitations of the appartus prevented the administration of higher integral dose rates. The combination of fenfluramine and an integral dose rate of 15 W produced a severe disruption in the behavior of three of the four subjects. One of the subjects did not respond at all under the combined drug-radiation treatment, while the other two subjects showed a 20 and 30 percent decline in response rates. One of these subjects displayed a nine percent decline in response accuracy, while accuracy of the other monkey declined 14 percent below control values. Range of variability across all other sessions was less than 5 percent.

DISCUSSION AND CONCLUSIONS

The lack of behavioral response to an intense microwave field is not in itself surprising. Relatively few studies have been reported using primate subjects, and the power levels required to produce a behavioral effect are typically higher than those sufficient for producing effects in smaller animals (see, e.g. 14,16,17,18).

Despite the fact that no microwave threshold dose was found, the combination of microwaves and a serotonin depleter resulted in a substantial behavioral deficit. In order to accurately determine if the drug and radiation interacted synergistically, additively, or if one potentiates the other, it would be necessary to combine several effective doses of radiation and drug. Since an effective dose of radiation was not found, such a determination is not possible at present.

The present data, along with the previous finding that PCPA and fenfluramine interact similarly with microwaves, suggest that microwaves interact with monaminergic process in the brain. Both fenfluramine and PCPA deplete monamines, primarily serotonin, without significant effect on acetlycholine (19).

Unfortunately the data do not suggest a site of action for microwave radiation. PCPA acts by blocking the synthesis of serotonin by inhibiting tryptophan hydroxalase, while fenfluramine acts by releasing serotonin from storage granules and probably by preventing its uptake (19). It is tempting to speculate that microwave radiation acts by enzyme inactivation, thereby reducing brain serotonin levels.

A variety of other alternatives exist, one of which would assume that microwaves, like fenfluramine, stimulates release of serotonin from storage granules, rendering it vulnerable to degradation.

These two hypotheses would lead to different predicted outcomes from an experiment in which an MAO inhibitor was administered immediately prior to irradiation. If the radiation stimulates serotonin release, its action would be largely blocked by MAO inhibition. On the other hand, MAO inhibitors would have a much smaller effect if serotonin was not being normally synthesized.

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OPEN DISCUSSION

DR. JUSTESEN: Did you say that there were no physiological reactions reported below 10 milliwatts per square centimeter?

DR. GALLOWAY: I said no morphological changes in neurons.

DR. JUSTESEN: Let me ask you, if you are willing to speculate, what one would be expecting of the tight junction associated with the blood brain barriers which seem to come down somewhere around 200 to 600 microwatts per square centimeter, one half hour presentation? I realize that this is speculative morphology because they have not firmly implicated the tight junction as being responsible. However, it is worthy of a guesstimate, anyway, isn't it?

DR. GALLOWAY: Yes, I would not consider the blood brain barrier as a morphological change. As you say that is speculative.

DR. JUSTESEN: I will not belabor the point because I am expecting that to be an important therapeutic modality one of these days, irrespective of what is producing it.

One other question: when you say that the forward power is 15 watts, is this the total that is going into the head?

DR. GALLOWAY: Yes, that is integral dose rate.

DR. JUSTESEN: Well, it is not a dose, because it normalized.

DR. GALLOWAY: It is the dose rate.

DR. JUSTESEN: Oh, you are talking about 15 watts per kilogram?

DR. GALLOWAY: Yes, 15 watts.

DR. JUSTESEN: Okay, then it is not a mass normalized dose.

DR. GALLOWAY: No.

DR. JUSTESEN: Then your monkey's head is about 1200 grams--1.2 kilo?

DR. GALLOWAY: Their brains are around 800 grams.

DR. JUSTESEN: I was thinking of the surrounding fat, bone, and so forth.

SESSION III. PHYSICAL MEASUREMENTS

Moderator: *Roger Schneider*

BROADBAND MEASUREMENTS OF DIELECTRIC PROPERTIES
Mays L. Swicord

QUANTITATION OF MICROWAVE BIOLOGICAL EFFECTS
Chung-Kwang Chou and Arthur W. Guy

NEAR-FIELD MEASUREMENTS OF RF FIELDS
Paul S. Ruggera

ELECTRIC FIELD MEASUREMENTS WITHIN BIOLOGICAL MEDIA
A. Y. Cheung

INTERNAL DOSIMETRY AND EXTERNAL MICROWAVE FIELD MEASUREMENTS USING MINIATURE ELECTRIC FIELD PROBES
Howard Bassen

INFRARED EMITTANCE OF ELECTROMAGNETIC PHANTOM MATERIALS AND INFRARED TRANSMITTANCE OF POLYETHYLENE
T. C. Cetas and G. Kantor

A FINITE ELEMENT TECHNIQUE FOR CALCULATING INDUCED INTERNAL FIELDS AND POWER DEPOSITION IN BIOLOGICAL MEDIA OF COMPLEX IRREGULAR GEOMETRY EXPOSED TO PLANE WAVE ELECTROMAGNETIC RADIATION
Stanley M. Neuder

COMPARISON OF CALCULATED ABSORBED DOSE RATE DISTRIBUTIONS IN PHANTOM HEADS EXPOSED TO 2450 MHz AND 915 MHz PLANE WAVE AND SLOT SOURCES
Henry S. Ho

CHANGE IN AVERAGE ABSORBED DOSE RATE ON A GROUP OF MICE UNDER REPEATED EXPOSURE TO 915 MHz MICROWAVE RADIATION
Henry S. Ho, Frieda Pinkavitch, and William P. Edwards

THE DESCRIPTION OF A SYSTEM TO IRRADIATE CELLS IN CULTURE WITH MICROWAVES
James R. Rabinowitz, Robert B. Olcerst, and W. W. Mumford

NEW TYPES OF MICROWAVE DIATHERMY APPLICATORS--COMPARISON OF PERFORMANCE WITH CONVENTIONAL TYPES
G. Kantor

BROADBAND MEASUREMENTS OF DIELECTRIC PROPERTIES

Mays L. Swicord
Division of Electronic Products
Bureau of Radiological Health, FDA

INTRODUCTION

Interest in low-level or athermal effects has increased considerably in the past few years. Of particular interest are the reported frequency-dependent and modulation-dependent biological effects. Such reports are perhaps more common in East European literature (1), but are not totally absent in the West with the work of Oscar (2) being an example. Frequency selectivity in most of the described experiments may imply resonant absorption on the molecular, cellular, or membrane level. The theoretical possibility of microwave absorption by DNA has been discussed by Eyster and Prohofsky (3). Enhanced effects due to modulation again possibly imply resonant processes in which perhaps a multiple photon process becomes more probable with increased amplitude or that increased Fourier components, the spreading in frequency due to contraction in the time domain, result in increased absorption at some frequency other than the fundamental.

Such possible absorption process should be able to be predicted by observed rapid frequency variations in the absorption constant. However, if one is looking for one event per cell, then the amount of absorption due to this resonant process may be insignificant compared to other absorption processes in the cell and/or medium, which means that one may have to isolate that portion of the cell system in order to observe selective absorption.

Normal spectroscopic methods for determining the absorption constant, i.e., determining the ratio of transmitted to incident energy, are relatively easy when dealing with electrically-thin samples (e.g., gases). Such methods have been tried on samples of tissue which, in most cases, have not been thin at microwave and millimeter wave frequencies with questionable results (4,5). The difficulty lies in separating the geometrical resonances of the sample and holder, which are of no interest, from variations in absorption due to the structure of the material itself.

A more appropriate quantity to be measured is the dielectric constant; appropriate for two reasons. First, most techniques of dielectric constant measurement inherently take into account the geometry and resulting resonances of the sample holder. In fact, such resonances are purposefully created. Secondly, the dielectric constant, from which the propagation constant can be determined, yields more information. Namely, the real as well as the imaginery part of the propagation constant.

Not only for the possible determination of selective absorption processes of various biomaterials but for convenience in routine dielectric constant measurements, a method suitable for measurement of complex dielectric constants over a frequency range of 0.5 to 18 GHz was developed. Dielectric constant values from 1 to 50 with a loss tangent of as much as 0.5 are measured with an accuracy dependent only on the sensitivity of the particular system used.

This method is particularly adaptable to use with automated systems but can also be used with slotted lines. A distinct advantage of this method is its independence of sample length and the easy construction of a sample holder. The method includes corrections for the sample holder.

BACKGROUND

Most methods used for dielectric measurements in the 0.5 to 18 GHz frequency range measure the impedance of a waveguide or transmission line loaded with the material in question. The shortcomings of such methods are that they:

a) require solution to transcendental equations resulting often in ambiguous results;

b) depend on the length of the sample often requiring accurate length measurements of small samples; and

c) may require different experimental configurations for each frequency of interest.

One of the most common impedance measuring methods is the short-circuited-waveguide technique. The general equation to be solved is the familiar transmission line equation

$$Z(\ell_3) = Z_3 \frac{Z(\ell_2) + Z_3(\mathrm{Tanh})\gamma_3 d_3}{Z_3 + Z(\ell_2)\mathrm{Tanh}\ \gamma_3 d_3} \qquad (1)$$

where Z_3 is the characteristic impedance of the line, $Z(\ell_2)$ is the impedance of the terminating load and γ_3 is the propagation constant. Figure 1 illustrates the physical situation.

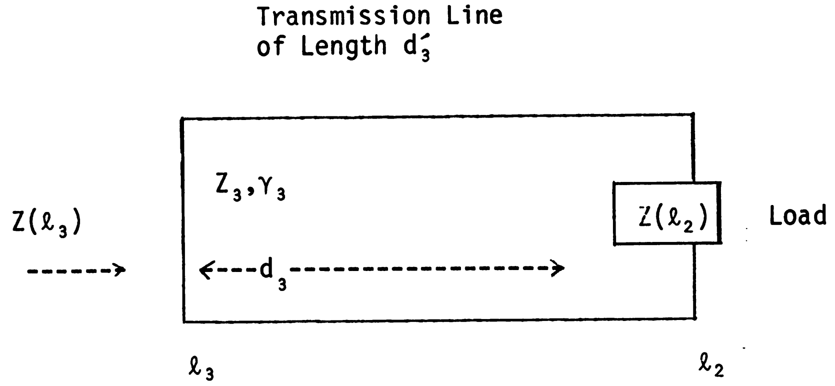


Figure 1. Transmission line terminated in load.

ℓ_3 is the location of the measurement reference plane and ℓ_2 is the location of the load.

If $Z(\ell_2)$ is set equal to zero (line terminated in a short, then $Z(\ell_3)$ becomes

$$Z(\ell_3^s) = Z_3 \text{ Tan } \gamma_3 d_3 \qquad (2)$$

Note that superscript s indicates a measurement with the line terminated in a short.

Since $\gamma Z = j\omega\mu$, then

$$Z(\ell_3^s) = Z_0 \frac{\gamma_0}{\gamma_3} \text{ Tanh}\gamma_3 d_3 \qquad (3)$$

where it is assumed that $\mu_3 = \mu_0$.

Equation 3 is normally solved for γ_3, often giving ambiguous results due to the multivalued solution of γ_3.

In addition, the method is sensitive to sample length and/or frequency. For example, the variation in node shift as a function of sample length for lengths greater than 1.5 wavelength and less than one wavelength is slight or unnoticeable (6). Thus, one must make measurements on an unknown sample as a function of sample length in order to find the region of sensitivity making swept frequency measurements impossible. For observation of resonant-type behavior, a method which is relatively independent of sample length and one which can be automated is desired.

Two Impedance Method

The transcendental nature of the transmission line equation can be eliminated if we note that the value of $\text{Tanh}\gamma_3 d_3$ has been determined by measurement. If a second non-zero terminating impedance, Z_L, is used at ℓ_2, then equation (1) combined with equation (2) becomes

$$Z(\ell_3^L) = Z_3 \frac{Z_L + Z(\ell_3^s)}{Z_3 + \frac{Z_L\, Z(\ell_3^s)}{Z_3}} \qquad (4)$$

Note that the superscript L has been used to designate a measurement with the line terminated in a load Z_L.

Equation (4) may now be solved for Z_3 in terms of the measured values of $Z(\ell_3^s)$ and $Z(\ell_3^L)$. The result is

$$(Z_3)^2 = \frac{Z_L\, Z(\ell_3^s)\, Z(\ell_3^L)}{Z_L + Z(\ell_3^s) - Z(\ell_3^L)} \qquad (5)$$

An added bonus is the independence of equation (5) of the sample thickness d.

Practical Considerations

Sample Holder

The construction of a sample holder which lends itself to the required measurements is not necessarily straightforward. First, all reflection coefficient measurements must be made with respect to the reference plane 3, requiring access of this plane.

A difficulty arises in using ℓ_3 as the initial reference plane, particularly for liquid or gel samples. A thin window can be used to define the location of ℓ_3. This, in conjunction with a sliding short inserted into the open line from ℓ_2, can be used to initialize a system using, for example a slotted line. However, an automated system such as a network analyzer requires several offset shorts for initialization. Under these conditions, the use of a thin window is impractical.

These difficulties are avoided by using a thick window. The experimental arrangement is diagrammed in figure 2, in which ℓ_3 is used as the initial reference plane.

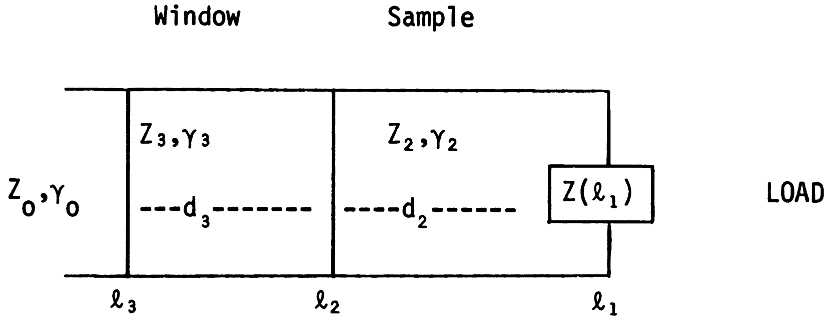


Figure 2. Schematic of sample holder using thick front window.

The characteristics of the window are first measured and determined using equations (2) and (5). The measured quantities $Z(\ell_3^S)$ and $Z(\ell_3^L)$ now determine the properties of the window.

The quantities $Z(\ell_2^S)$ and $Z(\ell_2^L)$ (not measured) are now defined as the impedance at ℓ_2 (front of sample) when the impedance of the line (end of sample at ℓ_1) is terminated in a short or load, respectively. The measured quantities $Z(\ell'_3)$ and $Z(\ell''_3)$ are now defined as:

$Z(\ell'_3)$ = Impedance observed at ℓ_3 when impedance at ℓ_2 is given by $Z(\ell_2^S)$.

$Z(\ell''_3)$ = Impedance observed at ℓ_3 when impedance at ℓ_2 is given by $Z(\ell_2^L)$.

The measurement with the sample terminated in a short results in equation (6).

$$Z(\ell'_3) = Z \frac{Z(\ell_2^S) + Z_3 \operatorname{Tanh}\gamma_3 d_3}{Z_3 + Z(\ell_2^S) \operatorname{Tanh}\gamma_3 d_3} \qquad (6)$$

$$= Z_3 \frac{Z(\ell_2^S) + Z(\ell_3^S)}{Z_3 + \dfrac{Z(\ell_2^S)\, Z(\ell_3^S)}{Z_3}}$$

This is solved for the value of $Z(\ell_2^S)$ in terms of measured quantities $Z(\ell'_3)$ and $Z(\ell_3^S)$ as

$$Z(\ell_2^S) = \frac{Z_3(Z(\ell'_3) - Z(\ell_3^S))}{Z_3 - \dfrac{Z(\ell'_3)\, Z(\ell_3^S)}{Z_3}} \qquad (7)$$

In a similar manner, $Z(\ell_2^L)$ can be determined from the measured quantities $Z(\ell_3'')$ and $Z(\ell_3^S)$ as

$$Z(\ell_2^L) = \frac{Z_3(Z(\ell_3'') - Z(\ell_3^S))}{Z_3 - \dfrac{Z(\ell_3'')\,Z(\ell_3^S)}{Z_3}} \tag{8}$$

Z_2 is now determined from equations (7) and (8) as

$$(Z_2)^2 = \frac{Z_L\, Z(\ell_2^S)\, Z(\ell_2^L)}{Z_L + Z(\ell_L^S) - Z(\ell_2^L)} \tag{9}$$

Liquid or Gel Samples

Liquid or gel-like dielectrics are easily measured using this method. A sample holder is constructed of one solid section, $d_2 + d_3$ (Fig. 2), with the window tightly fitting into the waveguide or coaxial holder a distance d_3. The line is then mounted in a vertical position. The surfaces ℓ_2 and ℓ_3 are well defined; the sample holder is removed for the initialization of the system at ℓ_3. The characteristics of the window can be measured using the two impedance methods by inserting a sliding short from ℓ_1 to ℓ_2 and determining $Z(\ell_3^S)$. $Z(\ell_3^L)$ is determined by placing a load at ℓ_1. A sliding load can be used to avoid the error of an imperfect load. Measurements are taken with the sliding load in three different positions, resulting in a circle in the complex impedance plane. The center of the circle is used as the value Z_L in equation (9).

Difficulty may arise due to a meniscus forming on the top liquid surface when the short is removed and replaced by a load. This problem is easily avoided by using a second window as shown in figure 3.

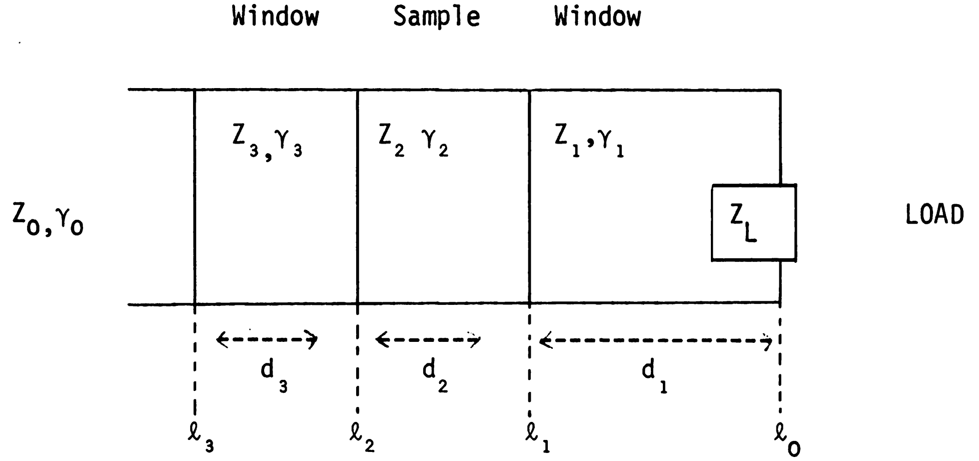


Figure 3. Sample holder with front and back window.

One additional measurement is then required to determine $Z(\ell_1^L)$. This is accomplished by treating the window and load as a new terminating impedance. The impedance of the new load $Z(\ell_1^L)$ is measured directly. Equation (9) then becomes

$$(Z_2)^2 = \frac{Z(\ell_1^L)\, Z(\ell_2^S)\, Z(\ell_2^L)}{Z(\ell_1^L) + Z(\ell_2^S) - Z(\ell_2^L)} \tag{10}$$

The impedance (Z) is related to the propagation constant γ by

$$Z = \frac{j\omega\mu}{\gamma} \tag{11}$$

where ω is the angular frequency and μ is the permeability. The measured quantity for most systems is the reflection coefficient ρ which is related to Z by

$$Z = Z_0 \frac{1 + \rho}{1 - \rho} \tag{12}$$

Where Z_0 is the characteristic impedance of the measurement system.

Thus the determined quantity is the normalized impedance Z/Z_0 which using equation (12) is given by

$$\left(\frac{Z}{Z_0}\right)^2 = \frac{\gamma_0}{\gamma} \tag{13}$$

The normalized impedance is related to the relative complex dielectric constant ε^* as

$$\left(\frac{\gamma_2}{\gamma_0}\right)^2 = \left(\frac{Z_0}{Z_2}\right)^2 = \frac{\varepsilon^*\omega^2 - \omega_c^2}{\omega^2 - \omega_c^2} \tag{14}$$

where ω_c is the angular cut off frequency. For a coaxial system, equation (14) reduces to

$$\left(\frac{Z_0}{Z}\right)^2 = \varepsilon^* \tag{15}$$

Limitations of the Method

Although length independence has been implied, this is not entirely true. A single sample length can be used over any given decade of the stated frequency range.

The limitation to a decade is best understood by considering the example plot of the reflection coefficient as a function of length of the sample as shown in figure 4. The example theoretical plots are for a dielectric constant of 50, and loss tangent of 0.4. The magnitude and phase are plotted for the sample terminated by a short and by a load. The variation of the reflection coefficient with length in units of wavelengths in the sample is insensitive below 1/8 of a wavelength and above 1-1/14 wavelengths.

The total useful frequency range is then constrained by the practical size of sample construction. Since samples can be constructed as thin as 2 mm, the upper practical frequency range is well above 18 GHz. Samples much longer than 20 cm are impractical. This would indicate a lower frequency limitation of about 200 MHz and possibly 500 MHz for most dielectrics of interest. Table 1 gives theoretical results of the variation of the reflection coefficient with dielectric constant, sample length and frequency and further illustrates the limitation of the method at lower frequencies.

Sample Holder Construction

Figure 5 shows a waveguide sample holder constructed for use at X-band frequencies. Plexiglas windows are used. The holder and front window can be constructed separately and fastened together as suggested by the view at the top of figure 5, or the section can be made of one solid waveguide piece and the window inserted. The back window is separate and can be fastened to the terminating load for measurement.

Care should be used in constructing and measuring the front window. An error in the measurement of Z_3 and $Z(\ell_3^s)$ will be amplified in the final results. For this reason, it is best to double check the window measurements by using a second window as a sample if possible.

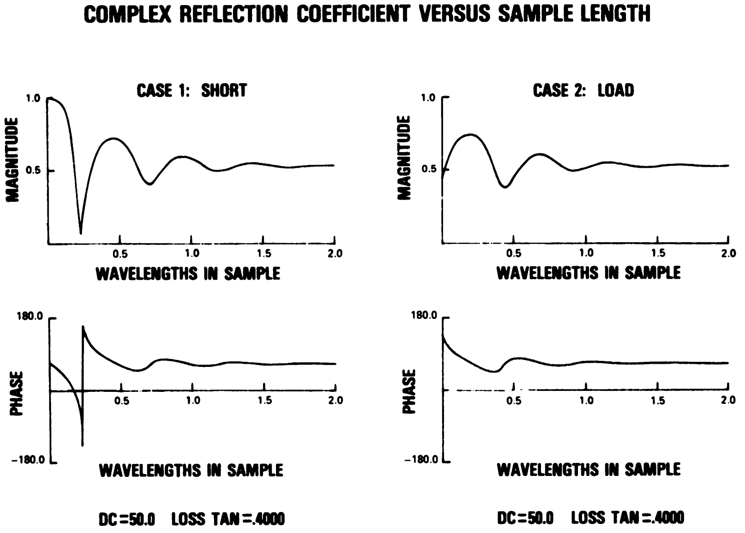


Figure 4. Complex reflection coefficient versus sample length.

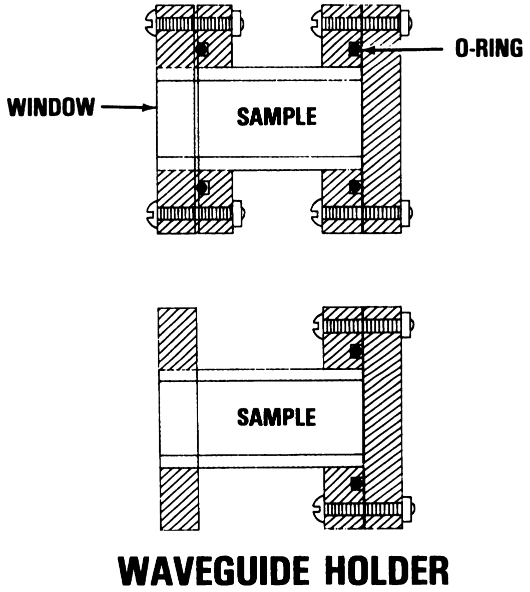


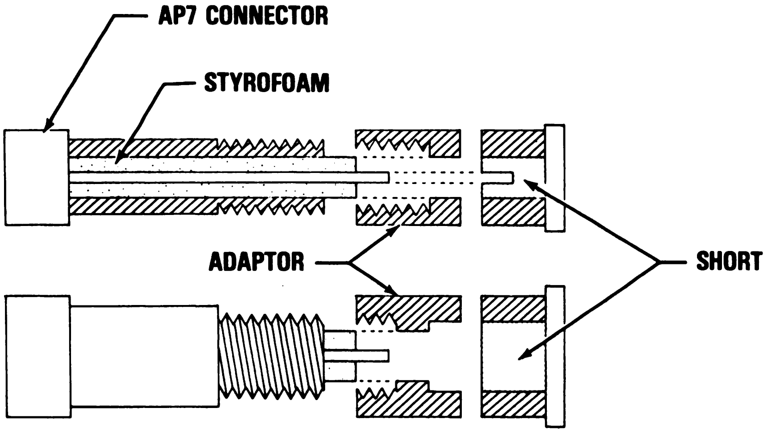
Figure 5. Waveguide holder.

Table 1. Measurement sensitivity of reflection coefficient

Frequency (MHz)	Dielectric Constant '	''	Sample Length (cm)	Ref. Coef. for Sample Terminated In Short 1	1	In Load 2	2
10	2.0	.01	20	1.00	175.1	.02273	-94.9
	1.9	.01		1.00	175.1	.02064	-94.9
	2.0	.02		1.00	175.1	.02273	-96.0
	50.0	.5		.99868	174.9	.62511	-153.1
100	2.0	.01	20	.99881	127.5	.20662	-128.7
	1.9	.01		.99888	127.8	.18867	-127.7
	2.0	.02		.99772	127.5	.20596	-129.7
	50.0	.5		.84759	177.5	.68694	172.7
300	2.0	.01	20	.97342	-42.2	.31297	162.5
	1.9	.01		.97453	-34.1	.29460	164.7
	2.0	.02		.94840	-42.2	.31068	161.8
	50.0	.5		.77286	173.2	.76554	172.7
300	2.0	.01	5	.99943	138.5	.19360	-124.1
	1.9	.01		.99945	138.6	.17972	-123.3
	2.0	.02		.99897	138.5	.19312	-124.9
	50.0	.5		.76734	-176.6	.82497	166.2
2500	2.0	.01	1	.90178	-105.3	.18569	-112.3
	1.9	.01		.88615	-85.3	.11438	-108.1
	2.0	.02		.84654	-105.6	.18209	-116.3
	50.0	.5		.56900	78.6	.56900	-18.6

Figure 6 shows the construction of a 7 mm coaxial sample holder. The 10 cm air line is filled with a high density polyurethane foam, leaving only a small portion of the line for sample containment.

The reference plane ℓ_3 is the unaltered end of the line. The window is then the full length of styrofoam. The reference plane ℓ_2 is the end of the polyurethane foam indicated in figure 6. The outer conductor of the end of the line is removable. Two different outer compactor adaptors are constructed so as to allow shorted measurements at ℓ_2 (top view Fig. 6), and shorted measurements at ℓ (bottom view Fig. 6). A special short is constructed which has a hole to allow clearance of the protruding center conductor for shorted measurements at ℓ_2.



AP7 COAX AIRLINE SAMPLE HOLDER

Figure 6. Coax airline sample holder.

RESULTS

Figures 7 and 8 show data taken on various samples using an automatic network analyzer. The dielectric constant (real part) is presented in figure 7 and the loss tangent is presented in figure 8.

Each point represents an average of three data points of consistent data. The values show agreement with slotted line data and expected results.

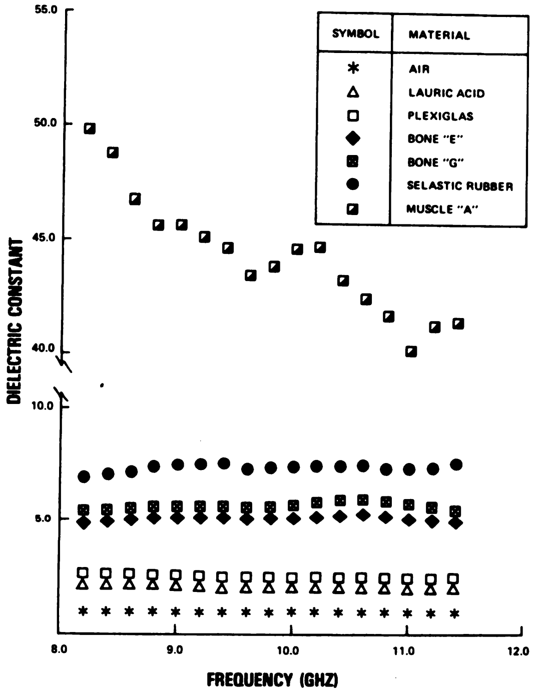


Figure 7. Dielectric constant of sample materials versus frequency.

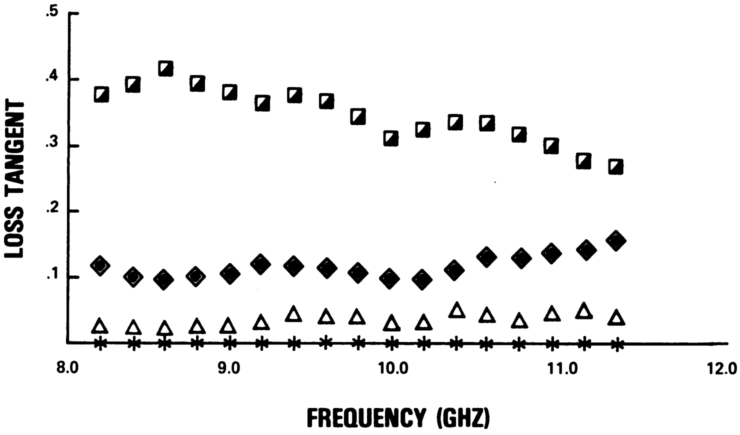


Figure 8. Loss tangent of sample materials versus frequency.

CONCLUSIONS

Physical measurements on biological systems are needed in order to predict the possible existence of frequency selective biological effects. The dielectric constant is one such possible parameter, and a method suitable for measurement from 0.5 to 18 GHz has been described.

The method avoids solutions to transcendental equations as well as the exact measurement of the sample thickness. The method of constructing a sample holder and exact method for correcting its presence in the system have been presented.

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QUANTITATION OF MICROWAVE BIOLOGICAL EFFECTS

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ABSTRACT

While emphasizing dosimetry and instrumentation, we have been able to demonstrate that the effects of acute exposure to CW microwaves on some of the electrophysiological properties of the nervous system are thermal in nature. Studies on the microwave auditory effect have provide strong evidence that the mechanisim of microwave hearing is electromechanical in nature. Development of a chronic exposure system and carbon EEG electrodes will provide a means for other researchers in pursuing the studies of biological effects of low level, chronic exposure of microwaves. In this reported research, the quantitation of microwave biological effects is stressed so that extrapolation to humans is possible.

INTRODUCTION

One of the problems in nonionizing radiation research is that the concepts and approaches used in ionizing radiation have often been incorrectly applied. Although the known biological effects of ionizing radiation are better understood than those of nonionizing radiation, the dosimetry problem of the latter is very different and much more difficult than the former. Unlike ionizing radiation, the same incident power density measured in the air can cause considerably different biological effects, presumably because of differential absorption which depends on the frequency, the configuration of source, the size and shape of object, the polarization of fields, the dielectric properties of tissues, etc. Although there are several thousand papers in the literature pertaining to the biological effects of microwaves, controversial results prevent any conclusions regarding the safety level of microwave exposure.

Since there are many variables determining the biological effects of microwaves, merely reporting the incident power density does not give enough information for comparisons between researchers using different animals and experimental approaches. Without quantitative measurements of the actual energy or field inside the exposed object, the value of specific research results is demeaned and limited to that animal or tissue exposed under that particular condition. If we are to gain in our knowledge, the following two steps are necessary to sufficiently quantify the biological effects of electromagnetic energy and to couple this data to our needs for promulgating rational human exposure standards: 1) determine what level of energy or power per unit mass absorbed in the tissues of an animal or specimen that will produce an effect, and 2) determine what level of incident power or field strength as measured by a survey meter will produce the same absorbed energy or power in the tissues of a human.

During the last 5 years, the Bioelectromagnetics Research Laboratory (BEMRL) has emphasized work on the quantification of microwave biological effects under the support of the Bureau of Radiological Health. In addition to the experimental development of quantitative methods involving thermograms and phantom materials, theoretical studies were also carried out for a better understanding of the relationships between frequency, dielectric properties, as well as the size and shape of objects. These theoretical studies were also essential for eventual extrapolation to humans. Several neurophysiological experiments were also carried out with special emphasis on delineating the mechanisms of the microwave effects obtained. Further, the level at which biological effects can be observed was always determined in terms of absorption in the tissue. Finally, through the use of thermography and phantom models of man, the energy absorption in man exposed to common sources was determined.

In addition, the problem of using metal electrodes for electrophysiological recordings during microwave exposure was demonstrated using thermographic methods. An alternative technique using the high resistance carbon electrodes has been develped in the BEMRL. The BEMRL has also developed techniques to chronically expose animals in a circularly polarized field at minimal cost. The laboratory has also worked on the development of safe methods and the evaluation of hazards for the application and monitoring of electromagnetic energy in diathermy practice.

This paper describes the approaches and findings of the research projects supported by the Bureau of Radiological Health and carried out in the BEMRL during the last 5 years. Previously published results will be described briefly. For more detailed information, the original references should be consulted.

METHODS AND RESULTS

Determination of Power Absorption in Spheres

Theoretical computations using spherical models and experimental studies on both phantom models and actual animals have shown theat mammalian heads exhibit resonance characteristics at certain microwave frequencies (1). The induced fields inside the brain may be an order of magnitude higher than those at the surface when the head is in a plane wave field. Extensive studies were made of the power deposition and distribution for spherical models of various size mammalian heads. Concentration of fields (hot spots) have been shown to exist in both human and animal heads. These theoretical results have been verified experimentally through phantom materials and thermographic techniques (2).

The peak and average absorption calculations for various size spheres over different frequencies indicate that the peak internal absorption for human head size spheres, i.e., resonance, is maximum in the UHF range centered around 1000 MHz (1). Power absorption patterns for more complex spherical structures, representing the head with 3 to 6 outer concentric layers of skin, muscle, bone, etc., indicate that these inhomogeneities have little effect on the internal power absorption pattern in brain tissue (3).

Effects of CW 918 MHz Microwaves on the Thalamic Evoked Responses

Tactile or auditory evoked responses at the thalamus region (VPL & medial geniculate) were recorded by Ringer's solution-filled glass pipette electrode which was fed through a low pass microwave filter to the recording instruments. CW 918 MHz microwaves at different power levels and durations were applied to the head region. The measurable effect of the microwaves appears to be an induced temperature rise in the thalamus region with an associated decrease in response latency (1,2). The threshold for both temperature rise and latency change was found to correspond to a maximum specific absorption rate (SAR) between 2.5 to 5 W/kg at the center of the cat brain. It would take a plane wave at an incident power density of 10 to 25 mW/cm^2 to produce the same SAR in the human brain.

The above latency decrease was duplicated using a heat exchanger embedded at the base of the skull circulating heated fluid (3,4). Conversely, it is possible to increase the latency by cooling the thalamus even during addition to microwave radiation.

Effects of CW 2450 MHz Microwaves on the Spinal Cord

A section of cat spinal cord at the lumbar-sacral region was exposed. Dorsal root was stimulated while the action potential was recorded at the ventral root. A Teflon ring was sutured to the dorsal skin to confine the Ringer's solution. A stipline applicator was used to irradiate the spinal cord. Monosynaptic responses were monitored before, during and after microwave exposure. Nothing in the results indicated that a given level of CW microwaves acts upon the spinal cord in any manner except to elevate the temperature of the tissue (3,4).

Effects of EM Field on Isolated Nerves and Superior Cervical Ganglia

The above in vivo studies involved exposures of whole animal heads and parts of the body to EM fields. These experiments cannot resolve with precise detail what portions or specific functions of the nervous system are being affected when exposed to EM fields. In vitro experiments with waveguide apparatus were designed to study the effects of microwaves on more isolated preparations. With isolated tissues, it is also possible to minimize the temperature effect by surrounding the exposed tissue with a temperature-controlled bathing solution. Since only small amounts of isolated tissue are pulled through the waveguide in regions of known field configuration and magnitude, quantitative dosimetry is easily achieved by mathematical calculations. Also, with the stimulating and recording electrodes outside the waveguide, artifacts due to field enhancements or EM field interaction with physiological amplifiers can be eliminated. In addition, the waveguide can simulate a whole range of low- to high-intensity exposure conditions.

Isolated frog sciatic nerves, cat saphenous nerves, as well as rabbit vagus nerves and superior cervical ganglia were stretched through the waveguide either parallel or perpendicular to the electrical field of the TE_{10} mode (5,6,7,8). Compound action potentials of nerves were recorded before, during and after the 2450 MHz microwave irradiation. When the temperature of tisues was kept at a constant level, results showed no significant change in the characteristics of nerves exposed to CW SAR of 0.3 -1500 W/kg and pulsed peak SAR of 0.3 - 220 kW/kg for pulses of 1 μs of 10 μs width. When the electrical current stimulation was turned off, no response was recorded during high-intensity microwave irradiation.

Effects of EM Fields on Isolated Muscle

By using the same waveguide, rat diaphragm muscles were isolated and exposed to 2450 MHz microwaves at SAR from 0.3 to 1500 W/kg for CW and pulsed waves of 0.3 to 220 kW/kg peak for pulses of 1 μs of 10 μs width (8,9). Results showed: 1) change in amplitude and time course of single twitch tensions as long as the temperature was kept constant; 2) at high power levels, the shortening of latency and decrease of amplitude can be produced by changing the solution temperature; 3) no effect on tetanic contraction; 4) no direct stimulation of muscle by microwave fields.

Effects of EM Fields on Auditory System

Perhaps the most widely observed and accepted biological effect of low average power EM energy is the auditory sensation evoked in man exposed to pulsed microwaves. This effect remained mysterious for about 15 years because of the proposed hypothesis of direct neural stimulation (10). Recent experiments in the BEMRL and other laboratories (11) have provided evidence that the hypothesis of electromechanical interaction due to the thermal expansion is the most likely mechanism of the microwave auditory effect. In addition to the finding of the microwave-induced cochlear microphonics, the sign of mechanical activation of cochlear hair cells, all the other results described in the following sections are consistent with the hypothesis of electromechanical interaction.

Determination of Thresholds for Microwave Evoked Responses in Humans and Cats

Thresholds of auditory sensation in humans and evoked responses in cats have been determined for microwave pulses 0.5 -32 μsec wide (12). It was found that regardless of the peak power and pulse width, the threshold was related to an energy density of 40 $\mu J/cm^2$ per pulse for cats. The peak absorbed energy density was calculated to be 16 mJ/kg in humans and was measured as 10 - 16 mJ/kg in cats. This energy density is capable of increasing the tissue temperature by only $5 \times 10^{-6°}$ C. As background noise (50 Hz to 15 kHz bandwidth) was increased, the threshold of evoked responses in the medical geniculate nucleus of the cat remained stable for pulsed microwave stimuli but increased for acoustic stimuli. This result indicates that the pulsed microwaves interact with the high frequency portion of the auditory system.

Measurement of Vibration of Lossy Dielectric Materials during Microwave Radiation

The displacements of the surface of lossy dielectric materials exposed to microwave pulses have been measured using the Michaelson interferometer. The pressures calculated from the measured displacements were close to that calculated from the thermal expansion theory (12).

Recording of Microwave-Induced Cochlear Microphonics

Bilateral cochlear destruction resulted in total loss of all evoked potentials due to microwave and acoustic stimuli (13). This result pointed out that the microwave auditory effect requires an intact cochlea. With proper recording techniques, we have recorded the microwave-induced cochlear microphonics (CM) from both guinea pigs and cats (14,15,16). These findings strongly support the hypothesis of electromechanical interaction and contradict the hypothesis of direct neural stimulation.

A CM of 50 kHz in frequency and 200 μs in duration was recorded from the round window of guinea pigs during irradiation by 918 MHz pulsed microwaves (14). Similar CM were also evoked mechanically by laser and piezoelectrical crystal pulses applied to the skull of guinea pigs (15). Lower frequency and shorter duration CM were recorded from cats (16). The latter finding raised the question of what determines the characteristics of CM. In order to answer the question, a series of experiments involving measurement of CM from guinea pigs and from cats of differing body mass during irradiation by 918 MHz and by 2450 MHz pulsed microwaves was carried out (17). Both horn applicators and a cylindrical waveguide exposure system were used to radiate the animals. The CM frequency and duration were similar, irrespective of carrier frequency and mode of application. Parameters of the CM (except amplitude) were not influenced by orientation of the body axis to the electrical field or by pulse width of microwaves for pulses less than 30 µsec wide. The frequency of CM correlated well with the longest dimension of the brain cavity and poorly with several other dimensions of the head. Extrapolations of these animal findings to human beings indicate that the frequency of microwave-induced CM in man should be between 7 and 10 kHz.

Surface-Recorded Evoked Response

Two carbon leads were attached to the skin of the quinea pig head at the vertex and the mastoid process (15). These leads were then connected to a Tektronix 3A9 differential amplifier. The output of the amplifier was fed to a computer of average transients. The averaged evoked potentials were plotted on an x-y plotter. Microwave pulses of 10 µs duration at intensities of 0 - 10 kW peak power per pulse were fed into the circular waveguide in which the head of the guinea pig was exposed. The repetition rates of the acoustic and microwave stimuli were 10 pps for brainstem-evoked responses and 1 pps for cortex-evoked responses. Similar brainstem or cortical level evoked responses were obtained during both acoustic and microwave stimulation. Masking noise of 2.5 kHz to 60 kHz depressed all the responses evoked by either acoustic or microwave stimualtion. These results show that after the electrical activity is activated at the level of hair cells, the neural transmission follows the same auditory pathway to the cortex.

Effect of Middle Ear Impairment on Microwave Hearing

In the discriminative control of behavior experiments (under separate funding) several rats, after extensive training, stopped responding to both microwave and acoustic stimuli. Surface electrode recordings showed no brainstem auditory-evoked responses. In all cases, severe otitis media was observed in these animals. At the time this suggested that the middle ear ossicular chain might play an important role in microwave hearing. To test this possiblity, 10 guinea pigs were used in a study where each animal's right cochlea was completely destroyed in order to prevent contralateral ear input. Brainstem auditory-evoked responses (BER) were recorded. Amplitude and latency intensity functions for maximum brainstem-evoked responses were obtained through both acoustic and microwave stimuli under different stimulus intensities. Figures 1 and 2 show that blocking the external auditory meatus affected the accoustic responses (down approximately 30 db) but not the microwave response. Filling the bulla with mineral oil also produced a similar effect on the two responses. Finally, destruction of the ossicular chain and the tympanic membrane caused severe hearing loss (down approximately 60 db) when stimulated by acoustic clicks. On the other hand, a much smaller effect was observed for the microwave responses. A similar effect was observed on the latency of the maximum brainstem-evoked responses. These findings argue against the notion that the middle ear bones are important for the microwave hearing effect, and is also consistent with the observation of Frey (18) the patients with conduction hearing loss can hear microwave pulses. When the cochlea was completely destroyed in the aforementioned guinea pigs, no response to either microwave or acoustic stimuli was recorded.

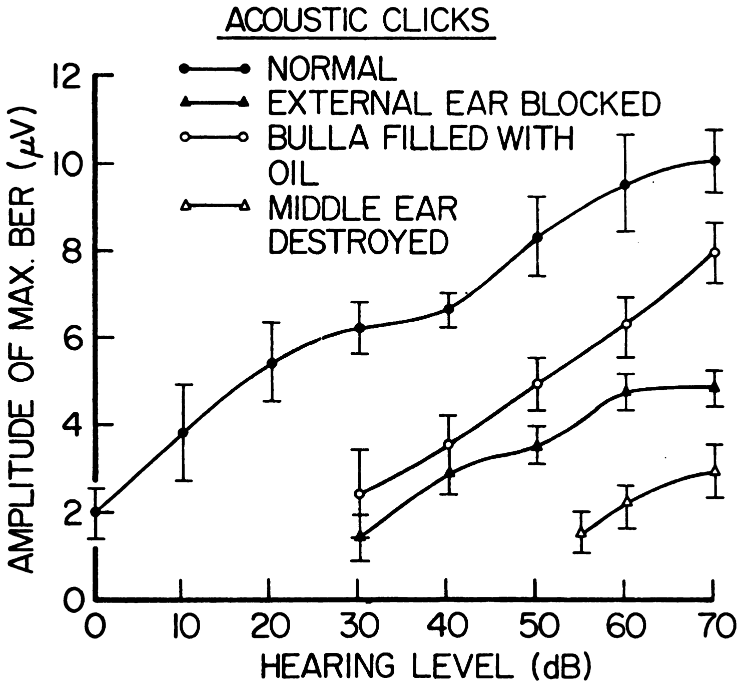


Figure 1. Effects of manipulations of external and middle ears on the amplitude of maximum brainstem evoked responses (BER) during 0.1 ms, 10 pps acoustic stimulation. (Subjects: five guinea pigs.)

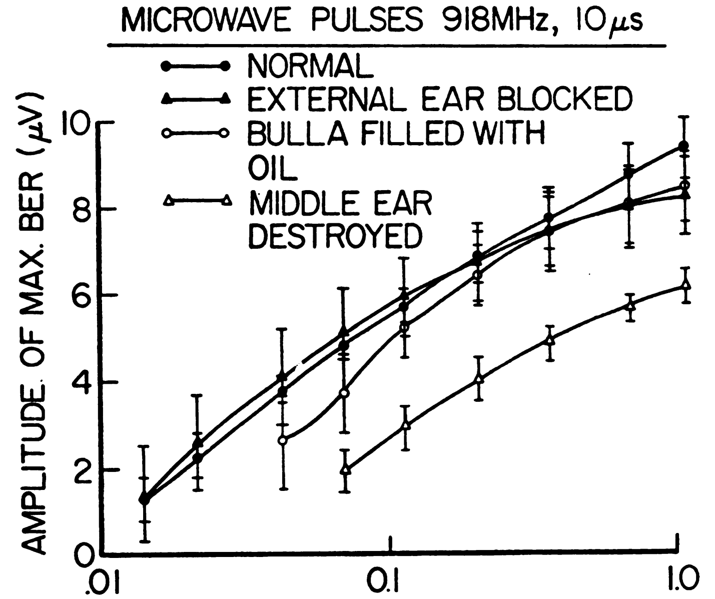


Figure 2. Effects of manipulations of external and middle ears on the amplitude of maximum brainstem evoked responses during 918 MHz, 10 μs, 10 pps pulsed microwave radiation.

Relationship of Pulse Width and Threshold Energy of Microwave Induced Hearing Response

Previous experiments in the BEMRL showed that the threshold of microwave auditory effect is related to the energy density per pulse and independent of the pulse width for pulses less than 30 μsec wide (12). The observation by Frey and Messenger showed that the loudness is dependent upon the peak power (19). According to the thermal expansion theory, the pressure generated by thermal expansion depends on the total energy at small pulse width and depends on the peak power at larger pulse width (8,11). According to Foster and Finch (11), based on theory, the transition occurs at the pulse width of 20 to 25 μsec. To verify this relationship between the pulse width and threshold of hearing, experiments employing a klystron generator were performed. The large klystron generator at the BEMRL can produce power up to 10 kW with variable pulse widths from 10 μsec to infinity in the frequency range from 720 to 1000 MHz. Using this generator and the cylindrical waveguide exposure system (14), the thresholds of brainstem-evoked responses were measured for pulse widths from 10 to 500 μsec. To avoid heating the head, pulses wider than 500 μsec were not used.

Figure 3 shows the relationship between the threshold absorbed energy density per pulse and the pulse width. For pulses less than 30 μsec, thresholds were at the same absorbed energy density or the same average incident power density. As the pulse width increased the threshold energy also increased. For pulses greater than 70 μsec, the threshold energy of the hearing effect on the pulse width agrees very well with the prediction of the thermal expansion theory. Our findings are also consistent with the observations of Frey and Messenger (19), although they interpreted their data differently. Their data showed, at the same averaged incident power density, that the loudness appeared the same for pulses shorter than 30 μsec and appeared less for pulses longer than 50 μsec.

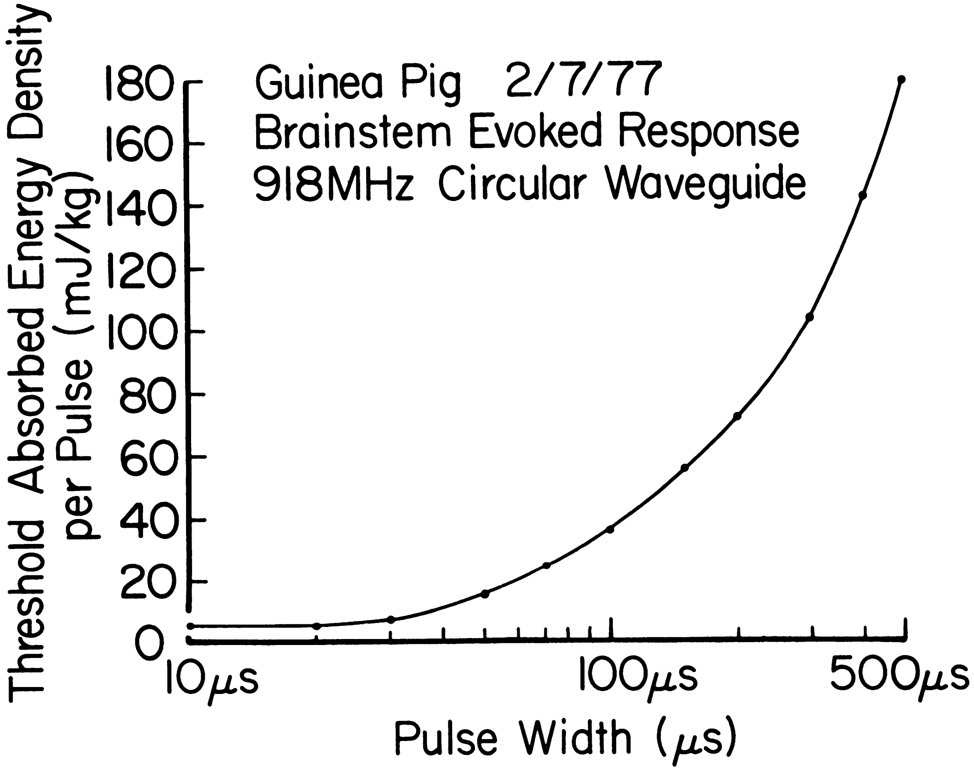


Figure 3. Variation of threshold absorbed energy density per pulse for microwave-induced auditory brainstem evoked responses at different pulse width.

Development of Dosimetric and Exposure Techniques

A system has been developed for economically exposing a large population of rodents on a long-term basis to 918 MHz electromagnetic fields without distrubing their normal laboratory living patterns. The system consists of a number of individual exposure cells connected through a power divider network to a single power source. Each cell consists of a section of circular waveguide constructed of low-cost galvanized wire screen in which a circularly polarized TE_{11} mode configuration is excited. Each cell contains a plastic chamber of adequate size to house a rodent up to the size of a guinea pig. The floor of the chamber, consisting of glass rods, allows waste to fall through plastic funnels into collection containers outside of the waveguide. The animal is fed from a special magazine containing dried food pellets that absorb very little of the microwave energy. The water supply is a standard laboratory animal's water bottle and glass nozzle except that it is electrically decoupled from the animal by two concentric one-quarter wavelength long coaxial choke sections. Each animal may move freely around in the chamber with minimal change in power coupling characteristics since the incident circularly polarized wave insures that the animal is uniformly illuminated with a propagating field (not unlike a radiation field), regardless of his orientation and movements. Severe changes in coupling due to animal orientation with respect to field polarization observed for plane wave exposure systems are virtually eliminated. The system allows easy quantitation of the average incident power density to the animal and the total power absorbed by the animal.

The maximum specific absorption rates (SAR) have been determined by thermographic studies on phantom models and on the actual bodies of sacrificed animals. Results show relatively constant power absorption for different orientations of the rats in the cell. Thermograms also show the effectiveness of the quarter-wavelength choke on the water bottle. Measurements were also made on the transmitted and reflected power from the cell over a 16-hour period for freely moving rats. For a 323 g rat, the net absorbed power was 43 percent ± 7 percent of the input power. The mean reflected power at the input terminal was less than 3 percent of the input power. A complete description of this chronic exposure system has been reported (20). Dosimetry measurement using rodents of different species and body weights are being carried out. Tabulated data will be available soon.

Development of Artifact-Free Electrodes for EEG Recording during Microwave Exposure

Johnson and Guy (1) have shown that a standard metal coaxial electrode normally used for neurophysiological experiments can produce very serious modification and intensification of the peak SAR by as much as two orders of magnitude. However, many investigators, both in the Soviet Union and in this country, are still using such electrodes in assessing the biological effects of microwave radiation on the CNS. Simply, there is no alternative way to do the experiments.

High-resistance carbon with conductivity close to tissue has been known to produce minimal effect on the field pattern in the tissue. With the Teflon carbon plastics (1-4 mhos/m) supplied by Mr. Ronald Bowman, of the National Bureau of Standards, we have developed electrodes useful for cortical or subcortical EEG recordings.

The cortical electrode consisted of a 6 mm long Teflon loaded carbon screw (either 2-56 or 4-40) and a fine carbon lead squeezed tightly by a nylon nut at one end. The other end of the lead was attached to a metal pin by conductive glue. The subcortical electrode was made of a glass pipette with tapered tip and a fine carbon lead inserted in the pipette with its tip extruding out of the pipette. The other end of the carbon lead was also glued to a metal pin. The resistances between the tips of all electrodes and the metal pins were less than 50 kΩ.

One rabbit (3.5 kg) was anesthetized with Nembutal (30 mg/kg IV). Atropine in the amount of 0.2 cc was injected subcutaneously before the anesthetic was administered. The rabbit head was fixed in a sterotaxic instrument. One carbon screw was placed at the sensory motor cortex. The other carbon screw, used as a reference electrode, was placed at the nasal bone area. The subcortical carbon electrode was inserted into the ventromedial hypothalamus according to the Atlas of Sawyer, et al. (21). A set of stainless screws and monopolar metal electrodes (Rhodes Medical Instrument SNEX 300) was also placed in the same location on the contralateral side. Cortical and subcortical EEG's were recorded from both types of electrodes. The EEG was amplified by a Tektronix 2A61 differential amplifier. The EEG spectrum was processed by a Tektronix 3L5 spectrum analyzer.

Figure 4 shows the EEG and spectrum recorded at the sensory motor cortex with both metal and carbon electrodes. Due to the high resistance of the carbon electrodes, contamination of high-frequency noise made the evaluation of raw EEG more difficult. However, the spectrums are similar for both types of electrodes with 60 cycle filter at position "in". It is impossible to record the EEG and spectrum from the carbon electrodes with the 60 cycle filter in "out" position. The effect of the 60 cycle filtering on the spectrum is shown at the left of figure 4. The major frequency components of the EEG were not affected by the filtering.

The subcortical EEG and its spectrum recorded with metal and carbon electrodes are shown in figure 5. These results also demonstrate the usefulness of the carbon electrodes.

In a separate experiment, a variable resistance of the 0 to 1 MΩ was connected in series with the metal cortical electrodes. The spectrum recorded with the 60 cycle filter in showed negligible change for the resistances used.

Three other rabbits with cortical carbon electrodes implanted showed no abnormality in a period of 6 months. One histological study showed good tissue compatibility of the carbon electrodes.

The above results indicate that the Teflon loaded carbon electrodes, which are transparent to microwaves, can be used for EEG recordings during the microwave exposure.

Therapeutic Heating Studies - Diathermy Safety

The problem of safety and dosimetry techniques associated with routine diathermy treatment with existing modalities has been evaluated using a 2450 MHz "C' director and a 918 MHz direct contact applicator (22). It was shown that the direct contact applicator does not only provide a solution to the safety problem, but also provides a better modality from the therapeutic standpoint. Moreover, with the direct contact applicator, it is feasible to use either forced air or circulating fluid surface cooling mechanisms which will further enhance the temperature increase in the muscle and consequently produce a more adequate therapeutic effect.

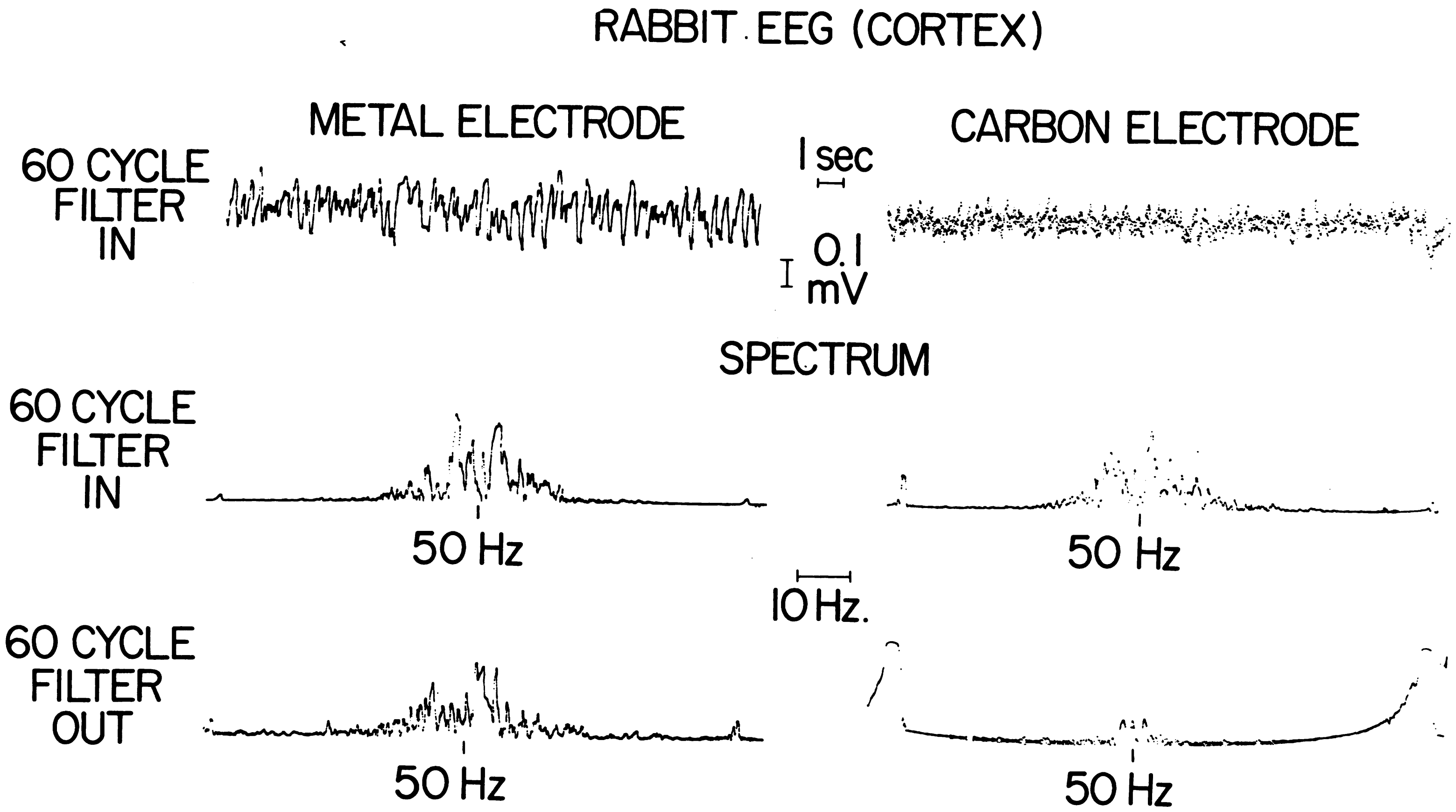


Figure 4. Comparisons of EEG and its spectrum recorded from sensory motor cortex of rabbit using metal and cabron.

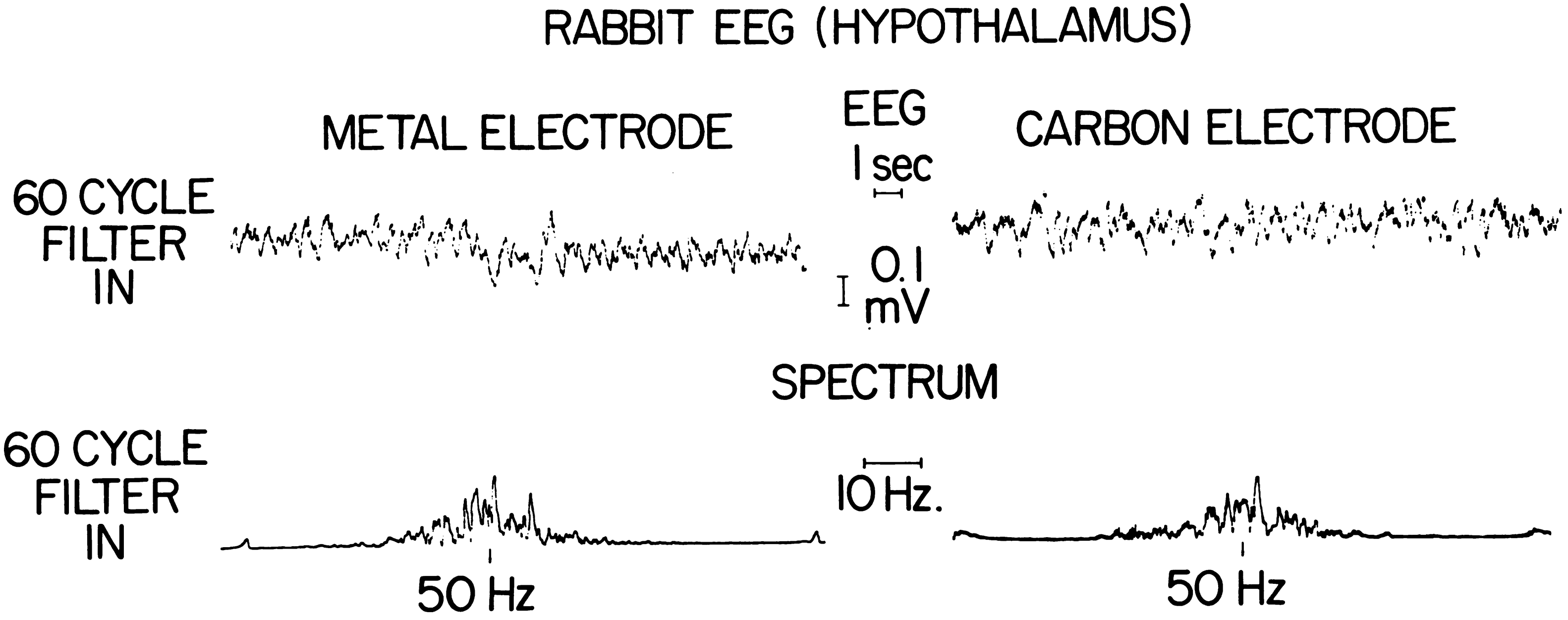


Figure 5. Comparisons of EEG and its spectrum recroded from ventromedial hypothalamus area of rabbit using metal and carbon electrodes.

The temperature rise in the human thigh during diathermy treatment with a 915 MHz air cooled contact applicator was measured by microwave transparent probes embedded at different depths in the tissue. For the measured temperature, the average peak SAR was calculated in the muscle as 120 W/kg and the blood flow was calculated on the average to be 27 ml/100 g•min.

Measurement of Specific Absorption Rate in Man Exposed to Electromagnetic Radiation

The above discussion related to the quantification of biological efects as measured in experimental animals. Regardless of how well this information is quantified, it cannot be extrapolated to man without knowing how electromagnetic fields from various sources couple to the body of man. The information needed is the specific absorption rate (SAR) patterns induced in man due to outside sources of interest in terms of safety. Our laboratory has developed techniques and facilites for exposing phantom scale models of man to high-power microwave radiation fields to simulate the exposure of full scale man to lower frequencies. After such exposures, the SAR patterns induced in the model can be determined thermographically. The present system utilizes a 10 kW CW 720-1000 MHz klystron source coupled to a 12' x 12' x 24' anechoic chamber. This allows the quantitation of the SAR patterns in man exposed to 80-250 MHz radiation fields which are most predominant in the environment of the general population. Typical patterns for the 198 MHz (Channel 11 television frequency) are shown in figures 6 through 10. SAR distribution measurements were made for all the eight primary orientations of the model man with respect to the incident fields. We may designate these primary polarizations using the nomenclature of Johnson et al. (23) by considering a coordinate system oriented with respect to the man with the x axis parallel to the long axis of the body, the y axis oriented parallel to the frontal plane, and the z axis oriented perpendicular to the frontal plane. Then we can define the polarization by which of the field vectors E,H,K are parallel to each of the axes, x, y, and z. Thus, EHK polarization is defined as the orientation for which E lies along x, H lies along y, and K lies along z. In addition, since a man is non-symmetrical, we must consider two additional primary polarizations over the six chosen by Johnson, et al. for the ellipsoid. In addition to the KEH and the KHE polarizations, where the head of the model is pointed toward the direction of propagation, we also must consider -KHE and the -KEH polarizations where the feet are in the direction of wave propagation. The mass, height, and scale factor of reduction for the model, as well as the frequency and the plane of observation, are shown at the top of each group of thermographic data. All the models were exposed to power densities of either 81 mW/cm^2 or 135 mW/cm^2, with applied powers of 3 kW or 5 kW, as indicated under each figure.

Exposure polarization is also indicated under each figure according to the convention discussed above. Intensity scans are shown at the upper left of each group of the thermographic data. They show the two-dimensional temperature distributions measured thermographically over the plane of separation for the particular model under study. The brightness of the intensity scan is proportional to temperature and a silhouette indicating the boundaries of the actual body has been scribed around each figure. At the upper right of each group of thermograms is a profile scan where multiple scans were taken over the surface of separation of the model. The scan consists of a two-dimensional distribution of measured temperatures with vertical deflection of each individual scan proportional to temperature. The E,H,K vectors are shown at the right of the intensity scan in their proper relationship with respect to the exposed subject. Below the intensity and profile scans are various double scans taken before and after exposure through the lines designated on the intensity scan. The vertical and horizontal scale of the intensity scan and the horizontal scale of the profile and double scans are 3 cm, division. The maximum SAR values

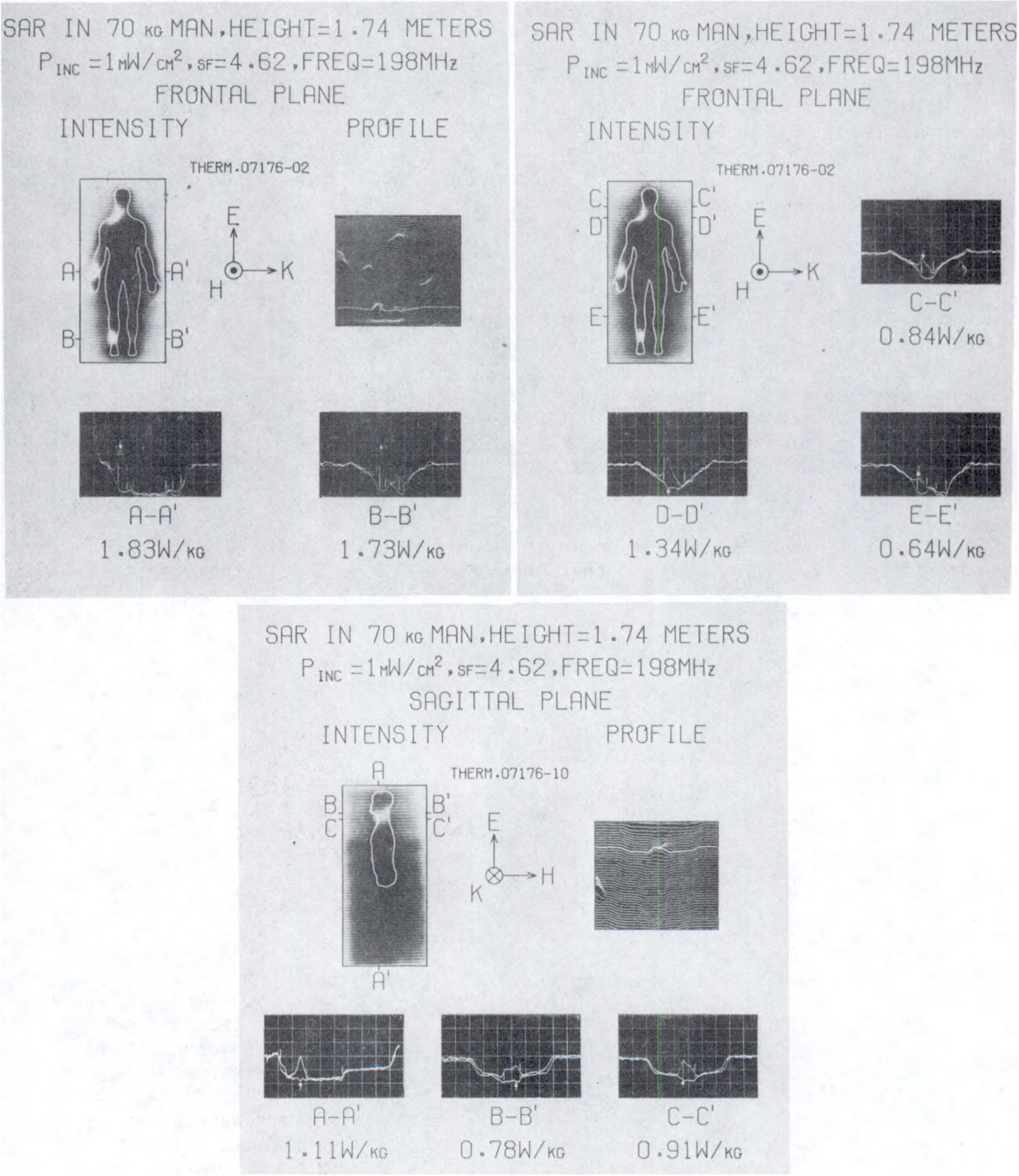


Figure 6. Measured SAR in man for EKH polarization at 198 MHz (test power 3 kW).

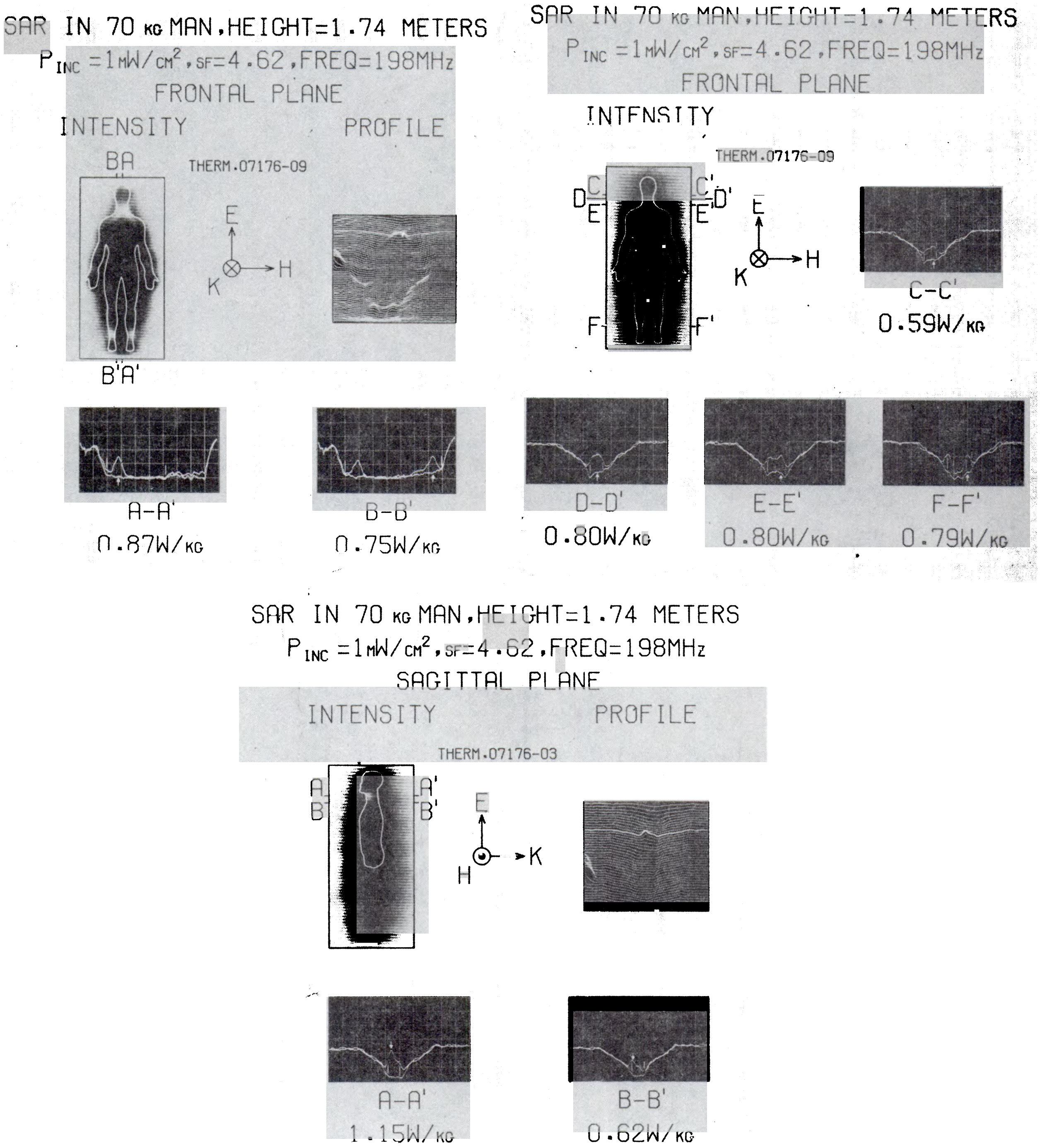


Figure 7. Measured SAR in man for EHK polarization at 198 MHz (test power 3 kW).

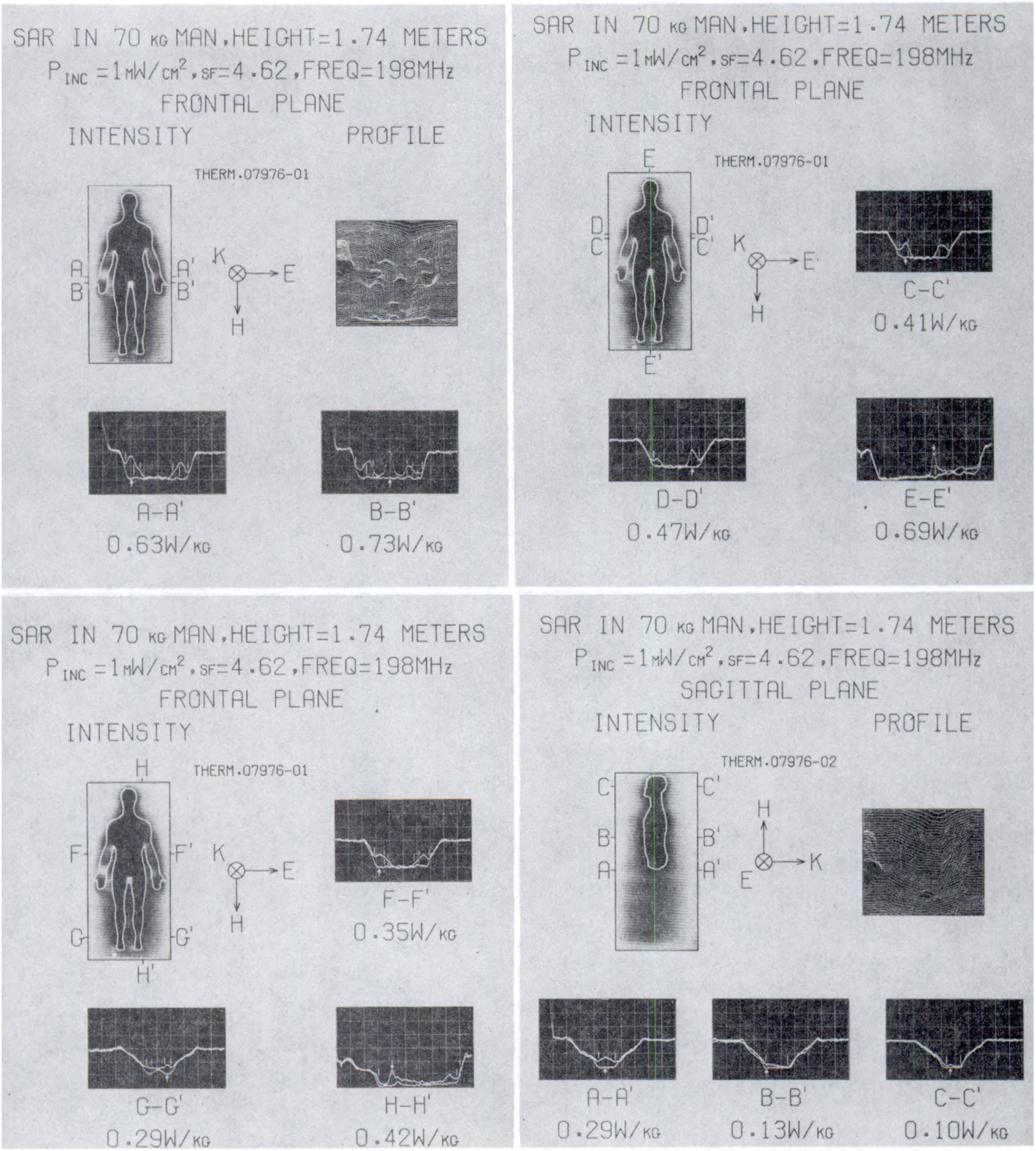


Figure 8. Measured SAR in man for HEK polarization at 198 MHz (test power 5 kW).

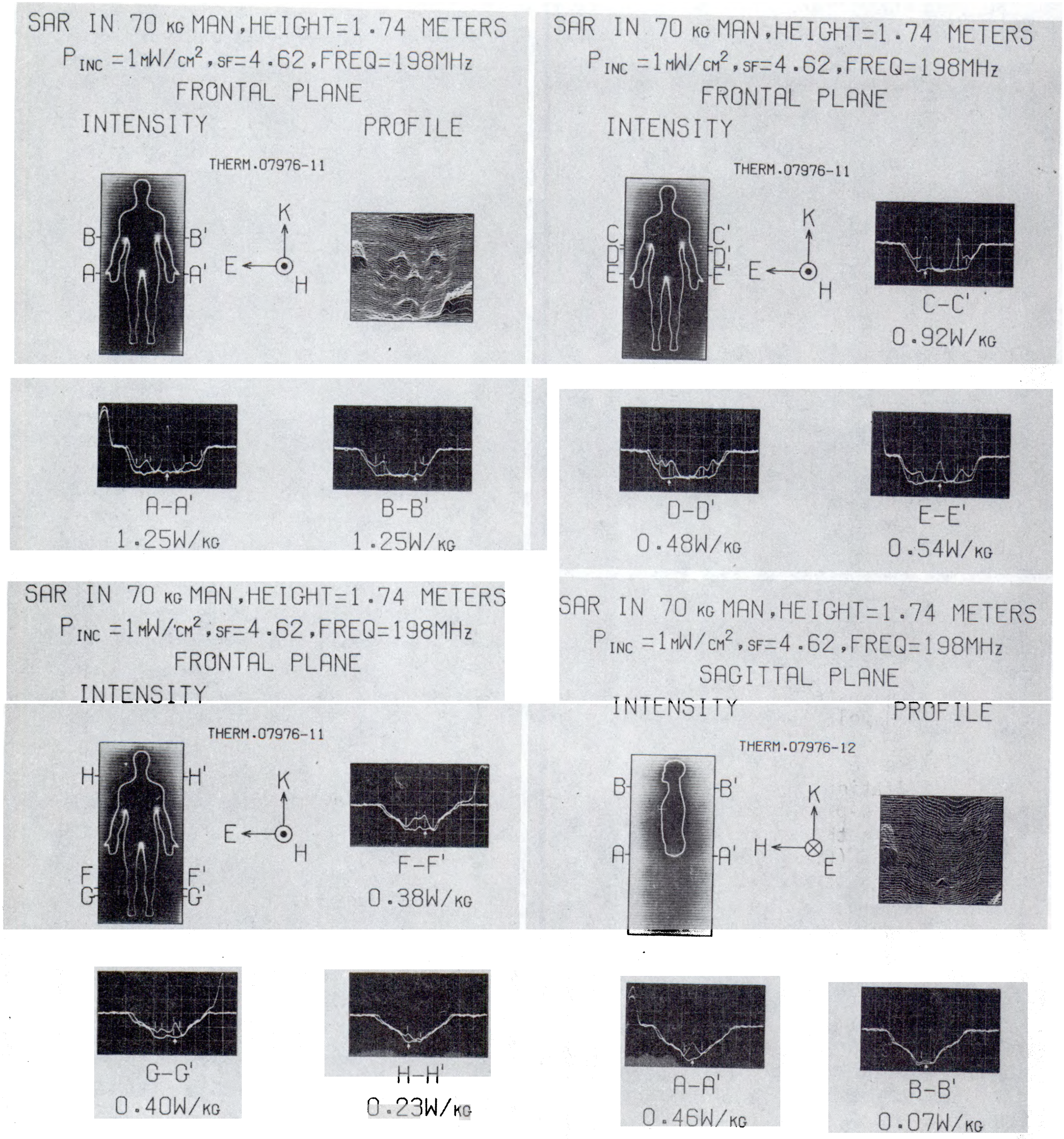


Figure 9. Measured SAR in man for KEH polarization at 198 MHz (test power 5 kW).

calculated from the temperature difference between the two superimposed profile scans are based on a normalized incident power density level of 1 mW/cm². Figure 6 illustrates the exposure of a 70 kg, 1.74 m high man to 198 MHz plane wave fields for EKH polarization (frequency near VHF television Channels 10 and 11). This exposure provided the highest measured SAR obtained for all exposures conducted in this study. The scans show that the highest SAR occurs in the neck, wrist, and ankle regions on the exposed side of the subject. Note that the peak SAR is 1.83 W/kg measured in the wrist. It is interesting to note that these regions of maximum measured SAR correspond to areas of the body with small cross sections. Thus, the longitudinal electric currents induced along the long axis of the body by the parallel electric field are being constricted by the small cross sections to produce high SAR's. One may also note that these maximum SAR's are more than an order of magnitude greater than the average values calculated for an equivalent prolate spheroidal model of the average man by Johnson, et al. (23). This would indicate that one should be very cautious about the use of the averaged SAR when considering possible thresholds of biological effects of man exposed to VHF radiation. Though the total absorbed energy or averaged SAR might be small in terms of the total body mass, the fact that this energy can be absorbed in a relatively small volume could lead to effects that would not normally be observed if the energy were distributed uniformly over the body. For example, based on the prolate spheroidal mode, an incident power density of 25-100 mW/cm² would produce from 2.75 - 11 W/kg average SAR in the body which is not too far from the threshold of biological and behavioral effects observed by many researchers. On the other hand, based on the measured SAR indicated in figure 6 the same incident exposure levels produce a peak SAR equivalent to a vigorous diathermy treatment. The level of absorption can result in localized temperature increases to 44-45° C after only minutes of exposure.

Figure 7 illustrates the case for the man being exposed to 198 MHz EHK polarization (man facing the source). In this case, the regions receiving the maximum SAR still correspond to the ankles, arms and the neck, while the right and left limbs experience the same SAR rate somewhat reduced from that observed for the EKH polarization.

Figure 8 illustrates the thermorgraphic results for the model exposed to HEK polarization. For this exposure condition where the man was facing the horn, (cross-polarized with the electric field), relatively intense hot spots were noted in the perineum area (0.73 W/kg), in the axilla area (0.41 W/kg), and in the wrists (0.63 W/kg). Again, these values of maximum SAR are more than an order of magnitude higher than that averaged value predicted from a prolate spheroid model. It is also interesting to note the unique specific absorption pattern with the maxima occurring in the tissue cleft regions. Similar type absorptions were noted for man exposed to magnetic fields with the magnetic vector perpendicular to the body. These two considerably different exposure situations do have common characteristcs, however. In both cases, electric fields are induced tangent to the apex of the tissue clefts. The presence of the high SAR in these regions can be explained from simple electromagnetic boundary conditions. If we consider a thin pill box-shaped void in a high dielectric constant medium and consider an applied internal electric field perpendicular to the large cross-sectional area of the void, the fields within the void will increase by a factor equal to the dielectric constant of the surrounding medium. This results from the continuity of the displacement vector through the void. At the narrow edge of the void, however, the parallel electric fields on each side of the interface must be equal. Thus, we would expect an intensification of the electric field in the tissue medium at this edge by the magnitude of the dielectric constant. This produces an SAR at the narrow edge that is many orders of magnitude higher than the average SAR surrounding the void. Another way to look at the phenomenon is to note that the void is interrupting the normal current flow over a relatively large cross section, forcing it to flow with increased density around the edges of the void. The sharp, angular change in the tissue in the axilla region and especially in the perineum region can produce this same efect. Note that for the sagittal plane, the measured peak SAR of 0.29 W/kg is less by a factor of 2 than measured for the same region in the frontal plane. This may indicate one of two possibilities: 1) heating of the adhesive due to the high electric fields in the foam near the apex of the perineum is contributing to the measured SAR pattern, or 2) the silk screen surfaces are not joined properly in the sagittal plane at the boundary region where the highest SAR values are expected.

When the man is realigned with the frontal plane still parallel to the electric field but the head in the direction of propagation (KEH polarization) the pattern is similar, as shown in figure 9. For this case, however, the peak SAR values are almost twice as high. When the man is re-oriented with the feet in the direction of polarization (-KEH polarization) the maximum SAR occurs in the limbs, as shown in figure 10. Table I summarized the maximum SAR measured for the various cases that were evaluated.

DISCUSSION AND CONCLUSIONS

Both in vivo and in vitro experiments have demonstrated that the effect of acute exposure to CW and/or pulsed microwaves on the electrophysiological properties of the nervous system considered in this study are thermal in nature. The threshold for a latency shift in thalamic evoked responses appears to fall between 2.5 - 5.0 W/kg SAR in the exposed tissues, which is about one-quarter to one-half of the normal metabolic rate of brain tissue. When extrapolated to humans, the incident power density required to produce an equivalent SAR in the head of man is between 10 to 25 mW/cm^2. When the temperature of the tissues was maintained at constant temperature during microwave exposure, no change in nerve action potentials or muscle contraction was observed.

Although at the threshold of the microwave auditory effect the temperature rise in the tissue increases by only 5 x 10^{-6}° C, this minute but fast temperature rise can generate thermal expansion pressure in the head, resulting in a hearing sensation. The cochlear microphonics data and the dependence of BER threshold on pulse width makes the hypothesis of electromechanical interaction the most likely mechanism of the microwave hearing effect. The studies on cochlear microphonics, middle ear and the brainstem-evoked responses demonstrate that the microwave pulses produce high-frequency mechanical distrubance to the hair cells in the cochlea and after this hair cell stimulation, the high-frequency click is perceived in the same manner as normal auditory stimulation. Further, the frequency of the click depends on skull size.

The development of the chronic exposure system has provided an economical method for exposing a large group of animals to quantifiable fields. Biological experiments (supported under separate funding) using this system have been carried out on a long-term basis. Over periods of months, no major problems were encountered and the system was found to function as well as originally anticipated. Measurements on power absorption for rodents of differing body mass are being carried out. Provided with this data, other investigators using this exposure system will be able to report their results in terms of accurate dosimetry.

Recordings of cortical and subcortical EEG using high-resistance carbon electrodes have been shown to be feasible and, although no thermographic study has been done as yet, the field concentration at the tip of the electrodes should be minimal.

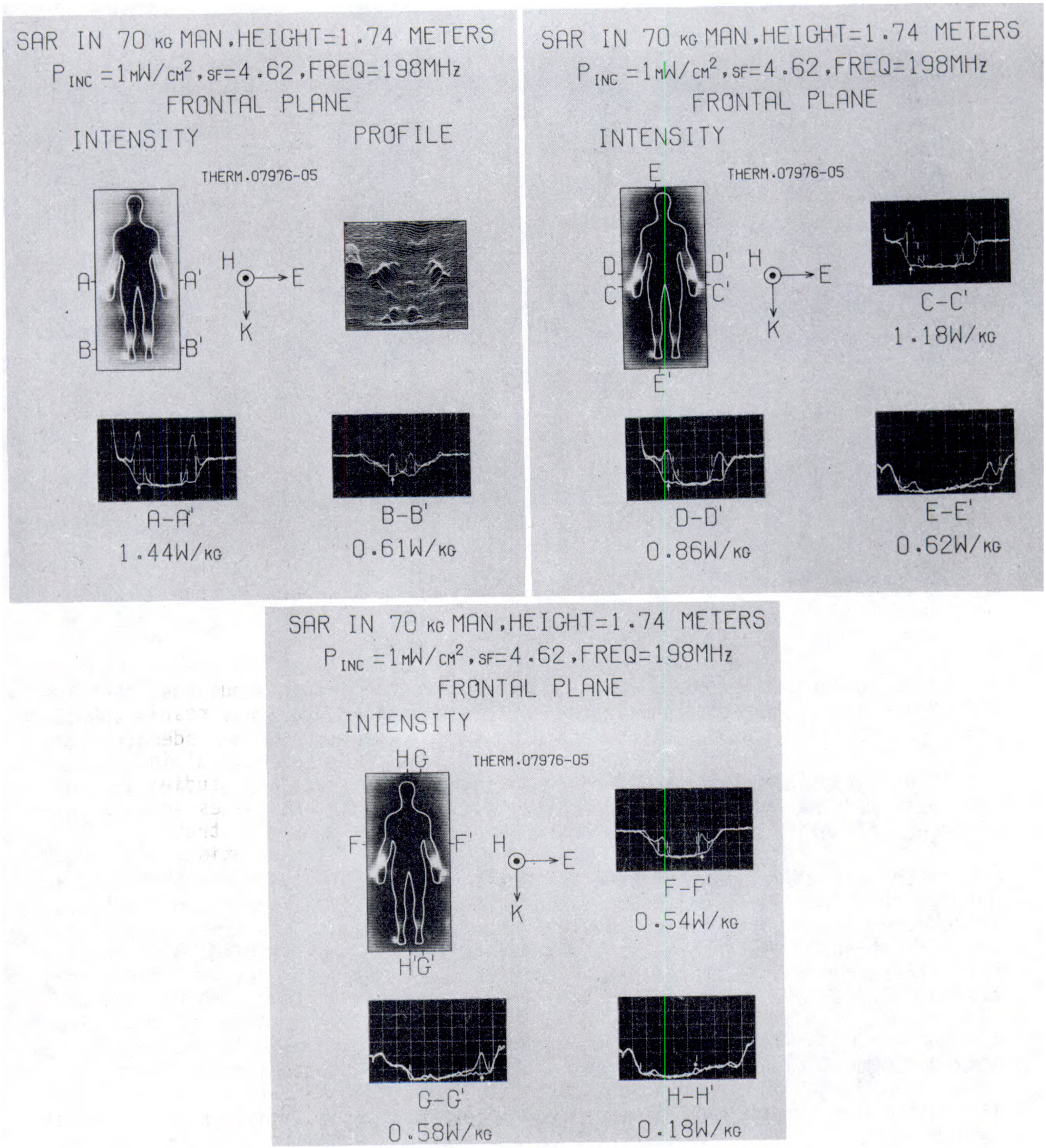
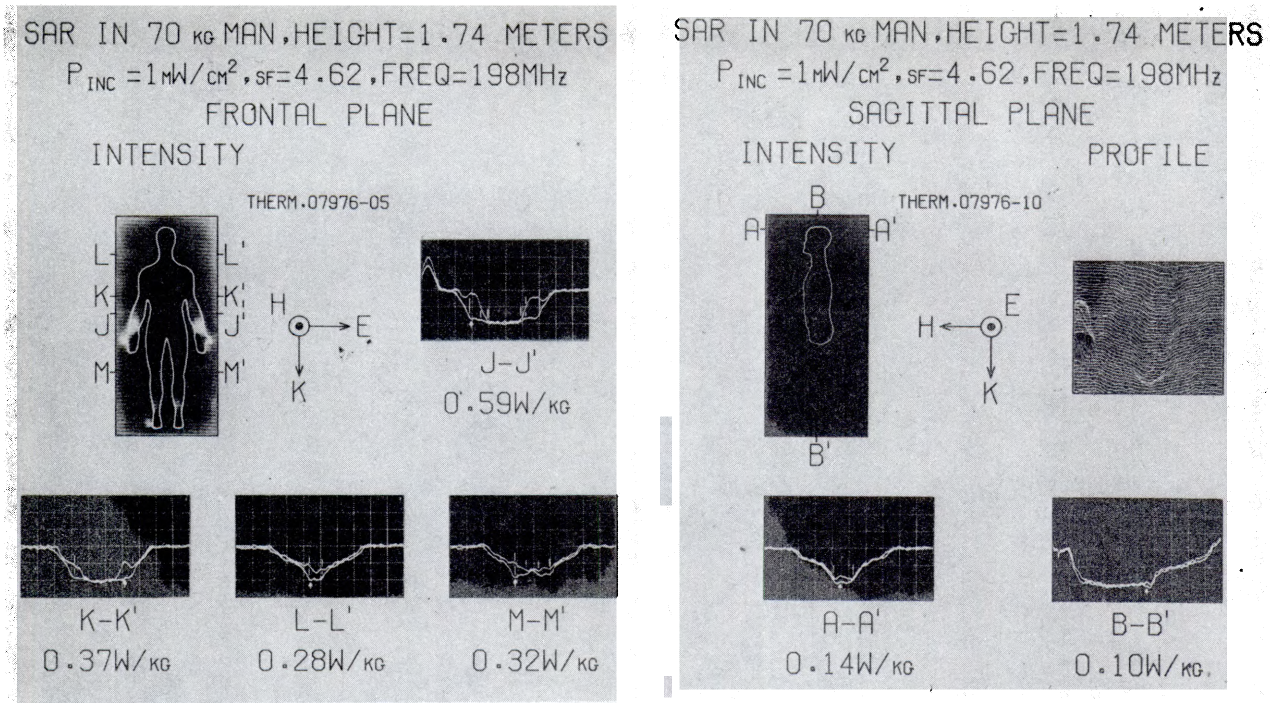


Figure 10. Measured SAR in man for -KEH polarization at 198 MHz (test power 5 kW).


SAR IN 70 KG MAN, HEIGHT=1.74 METERS
P_{INC} =1mW/cm², SF=4.62, FREQ=198MHz
FRONTAL PLANE
INTENSITY
THERM.07976-05
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K'
J
J'
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M'
H
E
K
J-J'
0.59W/KG
K-K'
0.37W/KG
L-L'
0.28W/KG
M-M'
0.32W/KG
SAR IN 70 KG MAN, HEIGHT=1.74 METERS
P_{INC} =1mW/cm², SF=4.62, FREQ=198MHz
SAGITTAL PLANE
INTENSITY
PROFILE
THERM.07976-10
B
A
A'
B'
H
E
K
A-A'
0.14W/KG
B-B'
0.10W/KG

Figure 10. (continued)

The studies on diathermy safety and applicator design have shown that the 918 MHz direct contact applicator not only can provide better therapeutic heating, but also produces less leakage than the existing 2450 MHz "C" director.

Thermographic measurements of man exposed to VHF frequencies indicate that peak-measured SAR values may exceed the estimated averaged values by more than an order of magnitude in various locations in the body.

Although all biological effects of microwave studies so far are thermal in nature, this does not lead us to exclude the possiblity of non-thermal effects. There are many reports on low level effects which are not explainable by simple tissue heating. Before the safety standard level can be settled, more quantitative research on the biological effects of electromagnetic waves is definitely needed.

ACKNOWLEDGEMENT

The major portion of this research was supported by the Bureau of Radiological Health, Food and Drug Administration Grant No. R01 FD 00646, and minor portions by the Rehabilitation Services Administration Grant No. 16-P-56818, and a NIH Fellowship Grant NIGMS-OADPA 1 F32 GM CA 05681-01.

Table 1. Summary of measured SAR in 70 kg man exposed to 198 MHz and 100 kg man exposed to 137 MHz radiation (values expressed in units of W/kg per 1 mW/cm^2 incident power density)

POLARIZATION	EKH		EHK		HKE		HEK		KEH	-KEH	KHE	-KHE
Frequency MHz	137	198	137	198	137	198	137	198	198	198	198	198
neck	0.51	1.11	0.51	1.15					0.23	0.28	0.16	0.26
wrist	0.41	1.83						0.63		1.44		
ankle	0.42	1.73		0.79	0.06			0.29	0.40	0.61	0.12	
knee	0.37	0.64								0.32		0.10
forearm	0.55		0.47									
perineum					0.17			0.73	1.25	0.18		
axilla								0.47	1.25	0.37		
arm			0.61					0.41		0.54		
head			0.15	0.59		0.29		0.10				0.19
shoulder	0.64	1.34										
chest						0.14		0.13				
hand					0.08							
shin									0.38			

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NEAR-FIELD MEASUREMENTS OF RF FIELDS

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ABSTRACT

The majority of electronic products that emit electromagnetic radiation (EM) to which the general public is inadvertently exposed operate at frequencies below 500 MHz. For the purposes of this paper, the radio frequency (RF) portion of the spectrum will be considered to extend from 1 MHz to 500 MHz. Some of the products for which no emission limits exist, or which are limited by the F.C.C. for reduction of their interference potential to communication systems are as follows: Citizen Band Radios; Induction Heaters (Sealers); Electrosurgical Units; Wireless Intercoms; Walkie-Talkies; Wireless Microphones; Security Transmitters; Intrusion Detectors; Field Disturbance Sensors; Door Opener Transmitters; Model Remote Control Transmitters; Amateur Radio Transmitters; Epilators; Electronic Games; Class I TV devices (couple to TV set with modulated RF oscillator); Master Antenna Systems; Ignition Systems; and Light Dimmers. Three of these exemplify the general problem and have been investigated by the Bureau of Radiological Health. These are: Radio Frequency Sealers; Electrosurgical Units, and Citizen Band Radios. A description of each of these, along with the data obtained in the near field are presented.

MATERIALS AND METHODS

Unlike analogous microwave frequency situations where it is usually sufficient to measure only the electric field (E), in the RF region, it is necessary to measure both the electric field (E), and the magnetic field (H). This is true because in the "near field," the ratio of E to H is neither constant nor equal to 377 ohms. To measure only one type of field and then mathematically transform its value to produce the equivalent power density--a term of more familiarity to biological researchers--is not correct. (In this paper that transformation has been made in some of the tables and graphs so that the "equivalent free space power density" indication will allow the reader some means of comparison to the measured E and H values presented.) In addition to this constraint, it is also necessary to account for polarization of the fields. Therefore, a multi-axis instrument, or means of obtaining the orientation of the field, is necessary. This measurement capability has been developed by the Bureau of Radiological Health and was used in these studies. {See Fig. 1 (E-Field) and Fig. 2. (H-Field)} The instruments and their calibration (traceable to the National Bureau of Standards) are described in detail in a paper entitled "E-and H- Field Instrumentation and Calibration Below 500 MHz"(1) and will not be discussed further in this paper.

RESULTS AND DISCUSSION

Radio Frequency Plastic Sealers

Radio frequency sealers are commercial devices that bond together two or more pieces of fabric or vinyl which ultimately become handles, pockets, or complete products such as golf bags through the simultaneous application of pressure and high radio frequency currents. Other versions are used to dry the glue in plywood and in ski manufacturing. Measurements have shown that employees could potentially be exposed to hundreds of mW/cm^2 (equivalent free space

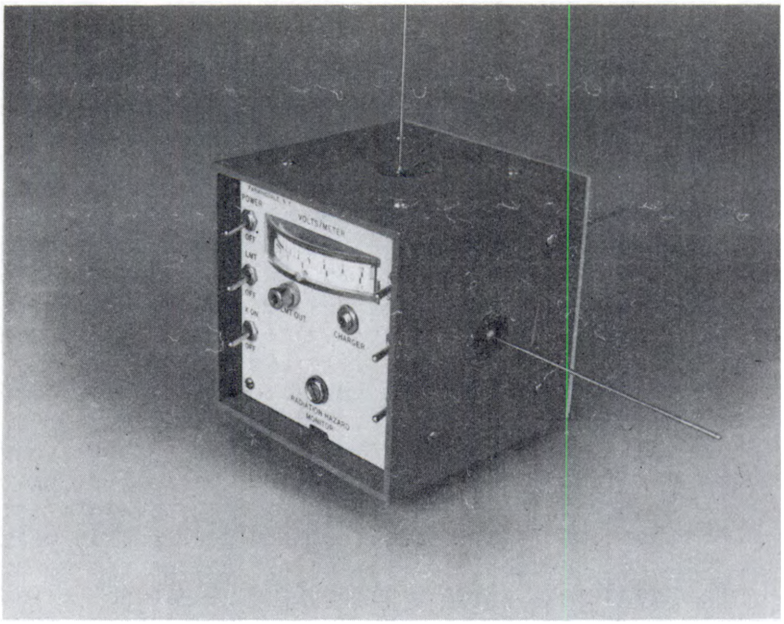
POWER
VOLTS/METER
CHARGER
RADIATION HAZARD MONITOR

Figure 1. E-field meter.

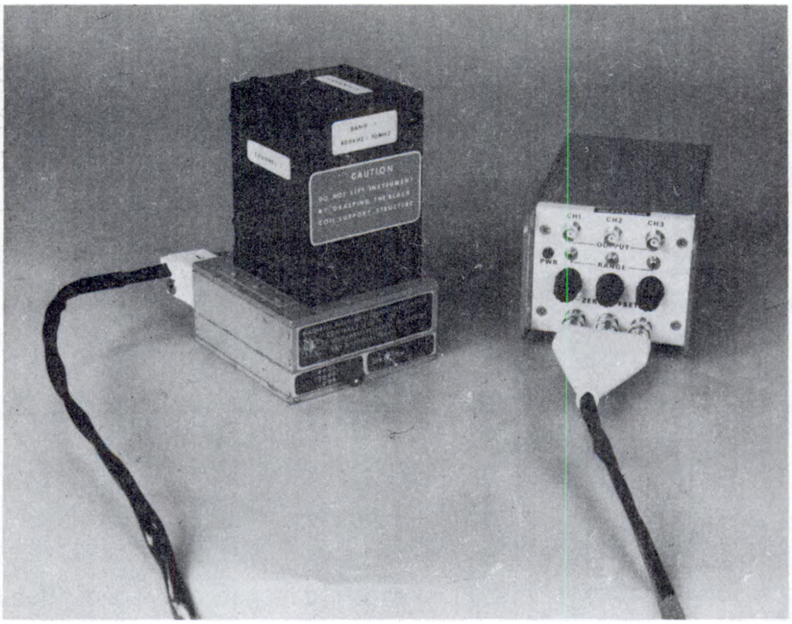
CAUTION
DO NOT LIFT INSTRUMENT BY GRASPING THE BLACK COIL SUPPORT STRUCTURE
CH1
CH2
CH3
OUTPUT
PWR
RANGE

Figure 2. H-field meter.

power density). These devices are not manufactured and delivered to the commercial user in such a way as to allow the machines to be installed and used without exposing the workers to high whole body radiation levels.

On May 3 and 4, 1976, measurements of electromagnetic emissions from several industrial RF sealers were made at a commercial plant in Portland, Oregon by BRH personnel. The effort concentrated on measuring the electric field with the 3-axis isotropic instrument developed for this frequency range. The magnetic field was also measured on one of the machines utilizing a single turn loop. Seven of the eight RF sealers were located in a rather loosely constructed (RF-wise) screen room. Complaints from communications users (police, fire, etc.) forced the construction of this room. The eighth, and newest, of the sealers was located out in the general work area, outside of the screen room. Figure 3 is a schematic of the plant's layout in the vicinity of the machines (about one-tenth of the total work area). The model, power output, and frequency of operation as obtained from the labels are also given. The units are numbered one through eight for easy reference to the technical information presented. Since the visit was made to assist OSHA, the main concern was measurement in the vicinity of the worker, and not a detailed evaluation of the actual source of emission, nor their absolute maximum amplitudes.

Figure 4 and Table 1 summarize the results of the measurements taken. The values have been transformed so that direct comparison with the equivalent free field power density can be made. In several instances, as shown, if the operator were to move from his "normal" 50 cm fixed position, exposure to the eye would certainly exceed the accepted 200 V/M or 0.5 A/M limit. In two instances, the field strengths exceed that limit at "normal eye position" (units 5 and 7). The magnetic field measured on unit 5 was extremely high.

A time analysis on one operator was also made (Unit 5). He produced a finished piece of a golf bag every 15 seconds. The RF was on for 2.5 seconds of this time; this results in the total exposure time of 10 minutes/hour. Figure 5 shows the operator's "normal" position using unit 4. Figure 6 is a close-up of unit 4.

Table 1. Field strength measured at location of the operator's eye in volts/meter and (equivalent free space power density)

Unit	V/M	Equivalent free space power density (mW/cm^2)
2	100	2.65
3*	100	2.65
4	70	1.3
6**	---	---
7	700	130.0
8*	120	3.8

*Unit not working properly.
**Unit broken.

Electrosurgical Units

The electrosurgical unit (ESU) is a device that is found in every operating room in the United States. It is also in many doctors' offices and in clinics. Basically, it is an electronic knife that has the capability of "cauterizing/coagulating" (stopping blood flow) in the process. It can be used for pure cutting, for pure coagulation or any combination of the two. Its frequency of

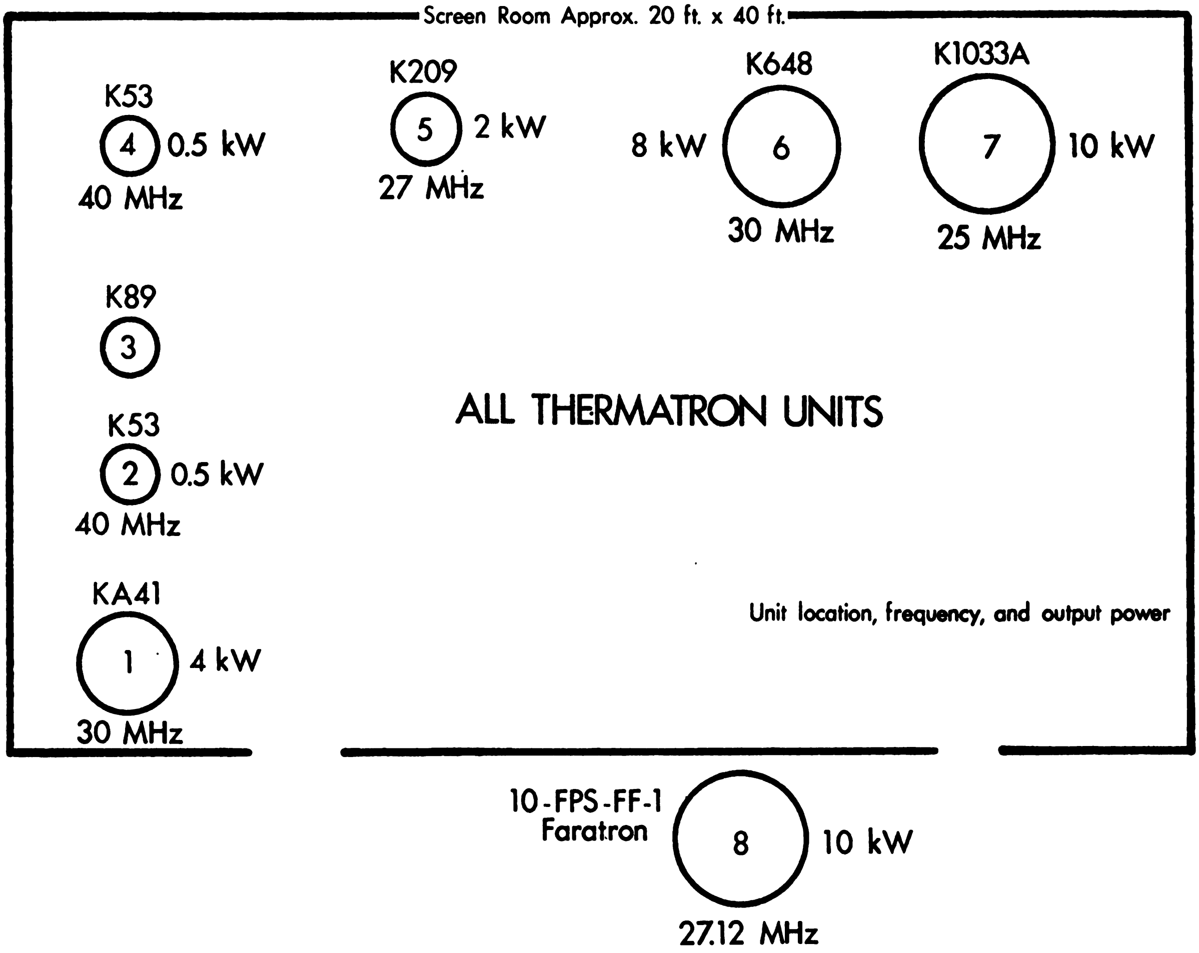
Screen Room Approx. 20 ft. x 40 ft.
K53
4
0.5 kW
40 MHz
K209
5
2 kW
27 MHz
K648
8 kW
6
30 MHz
K1033A
7
10 kW
25 MHz
K89
3
ALL THERMATRON UNITS
K53
2
0.5 kW
40 MHz
Unit location, frequency, and output power
KA41
1
4 kW
30 MHz
10-FPS-FF-1
Faratron
8
10 kW
27.12 MHz

Figure 3. Schematic of floor plan containing RF sealers.

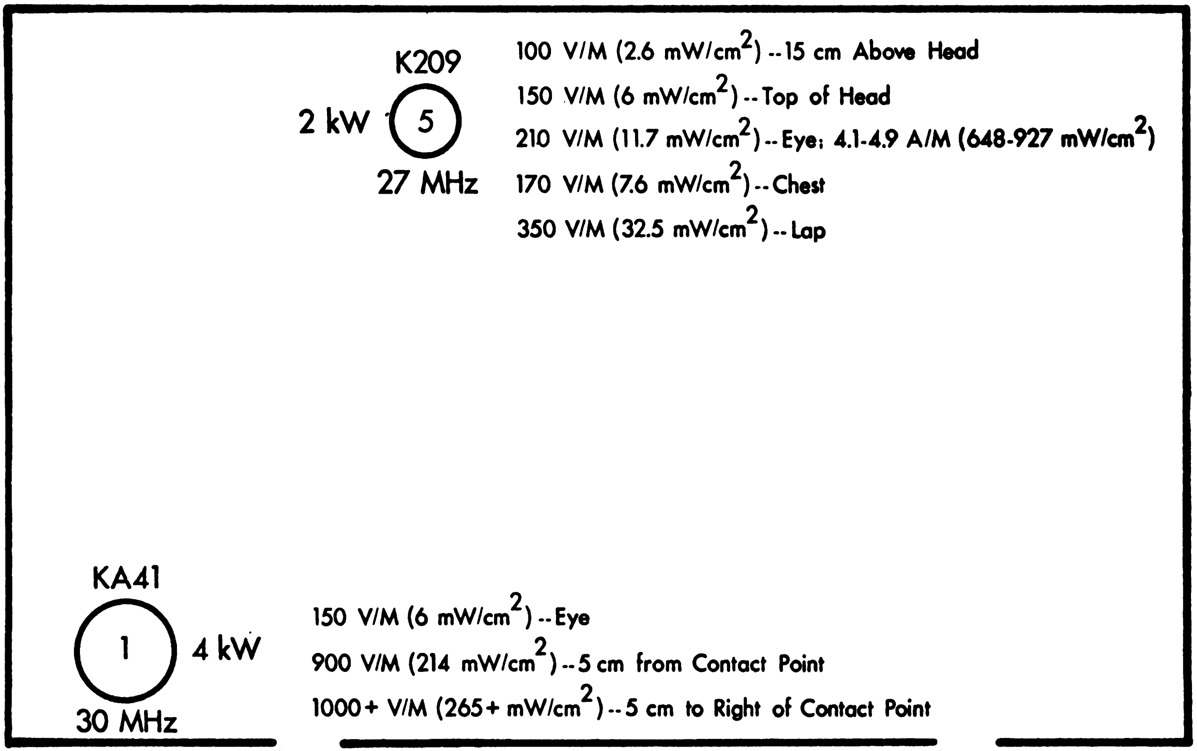
K209
2 kW
5
27 MHz
100 V/M (2.6 mW/cm^2) -- 15 cm Above Head
150 V/M (6 mW/cm^2) -- Top of Head
210 V/M (11.7 mW/cm^2) -- Eye; 4.1-4.9 A/M (648-927 mW/cm^2)
170 V/M (7.6 mW/cm^2) -- Chest
350 V/M (32.5 mW/cm^2) -- Lap
KA41
1
4 kW
30 MHz
150 V/M (6 mW/cm^2) -- Eye
900 V/M (214 mW/cm^2) -- 5 cm from Contact Point
1000+ V/M (265+ mW/cm^2) -- 5 cm to Right of Contact Point

Figure 4. Measurement of selected RF sealers.

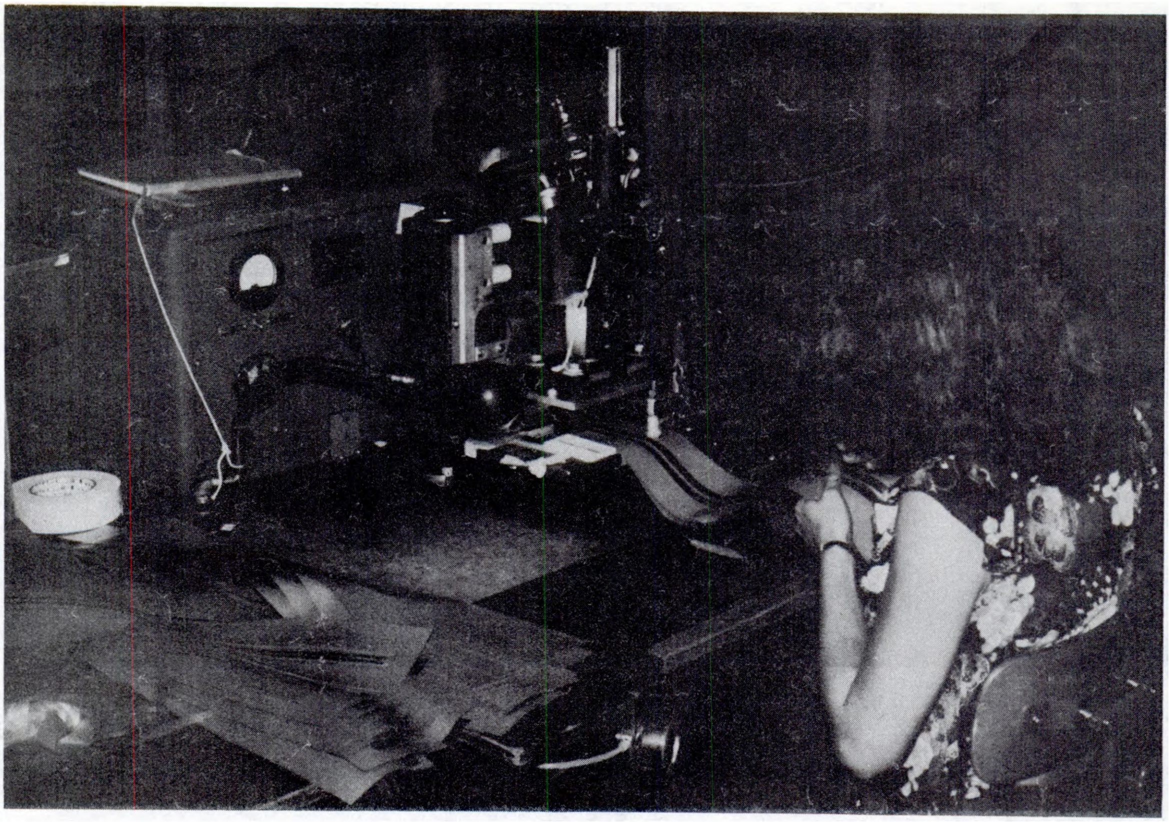

Figure 5. Normal position of operator of RF sealer.

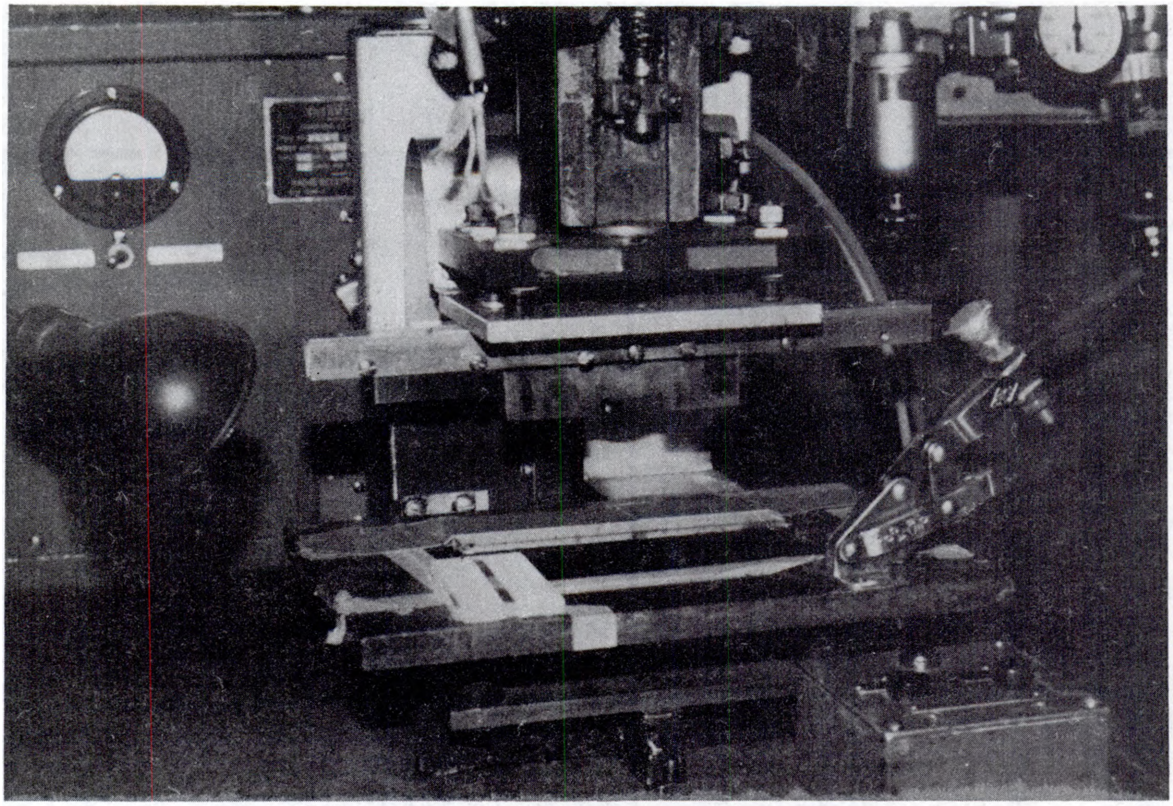

Figure 6. Close-up view of typical RF sealer.

operation is from 0.5 to 2.4 MHz continuous wave (cw) for pure cutting; an amplitude modulation of the cw (in the region of 10's of kilohertz) being added when coagulation is desired. In the coagulation mode the RF energy is interrupted at periodic intervals, the tissue then has a chance to cool and, thus, "coagulate." The principle of this device is that 4,000 to 8,000 volts (depending on the model) are applied to the cell. This raises the water in the cell to boiling point very quickly, bursts the cell open, and the tissue is cut. The "blade" of the electrosurgical knife is connected to the generator by an unshielded, but insulated wire. The return path from the knife is through the patient's body to a dispersive plate, usually placed on a large area of the patient's body--such as the buttocks. The plate is coated with a condutive "gel" to minimize the possibility of hot spots (radio frequency burns).

Although their principal frequency of operation is around 2 MHz, due to the "antiquated" design--dating back to the 1920's--the electrosurgical units emit frequencies up to 100 MHz. This device has been studied briefly in BRH laboratories. At 16 cm distance, (see Fig. 7) equivalent power densities of 265 mW/cm^2 were measured under maximum control settings. Under "typical use conditions," as recommended by the user's manual, 100 mW/cm^2 were measured. These fields may not only be a potential health hazard because of personnel exposure but are also a potential hazard resulting from EM interference (which is well documented in the literature) with other electronic medical devices such as patient monitoring and life support equipment.

On August 18, 1976, the emission patterns from the active electrodes of the ESU were measured. The primary E field, up to a meter from the source, was radially directed from the probe lead and, similarly, the primary H field direction was circular around the lead. This is depicted in figure 8. During the active portions of the measurement, a piece of flank steak was "operated" on. The field strength instruments were positioned 16 cm from the active lead, which was the minimum distance at which the maximum reading of the BRH E-field meter (1000 V/m) would not be exceeded. Both E and H measurements were performed under three basic modes of operation at normal and maximum power output, and were recorded for both ESUs. Tables 2 and 3 present the normal settings recommended by the manufacturer with the fourth entry representing one maximum condition. The far field equivalent power density ($E^2/3770$ in the table) is presented in the second column. The maximum measured was from the solid state unit in Table 2 and was 265 mW/cm^2 at 16 cm.

Briefly, the tables present the data as follows:

The E (V/m) column is the actual reading of the radial component of the electric field read on the instrument in volts per meter at the 16 cm distance.

The $E^2/3770$ reading is that which would be measured in mW/cm^2 if a power density meter with proper orientation and proper correction factor were used.

The H (A/m) column is the reading of the circular component of the magnetic field, in Amperes per meter, surrounding the lead at the 16 cm distance.

The E x H column is the magnitude in mW/cm^2 if the E field and the H field are multiplied together.

Additionally, a continuum plot was made about the 16-cm test distance to verify that by some circumstances a peak intensity region, due to standing waves, had not been located at the test distance. The data about the 16-cm test point was taken using a positioning system which allowed precise measurements to be recorded as the distance from the source was increased to approximately 1 meter (Fig. 9). Figure 10 shows the result of that test. As can be seen by the

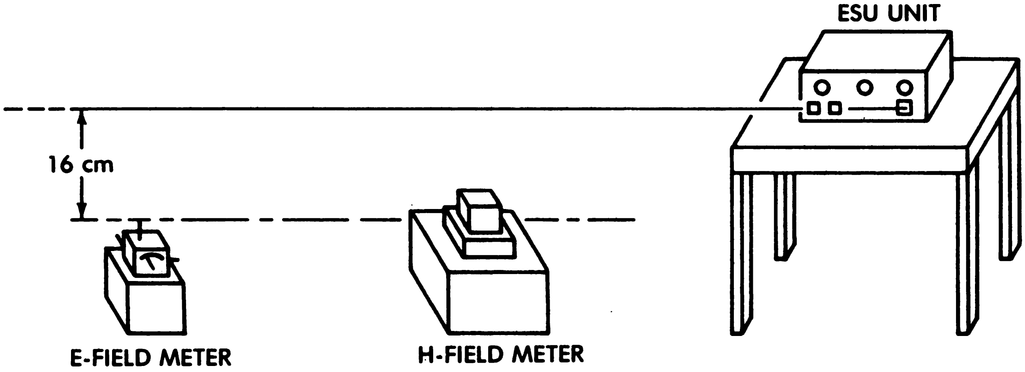
ESU UNIT
16 cm
E-FIELD METER
H-FIELD METER

Figure 7. E and H field measurement.

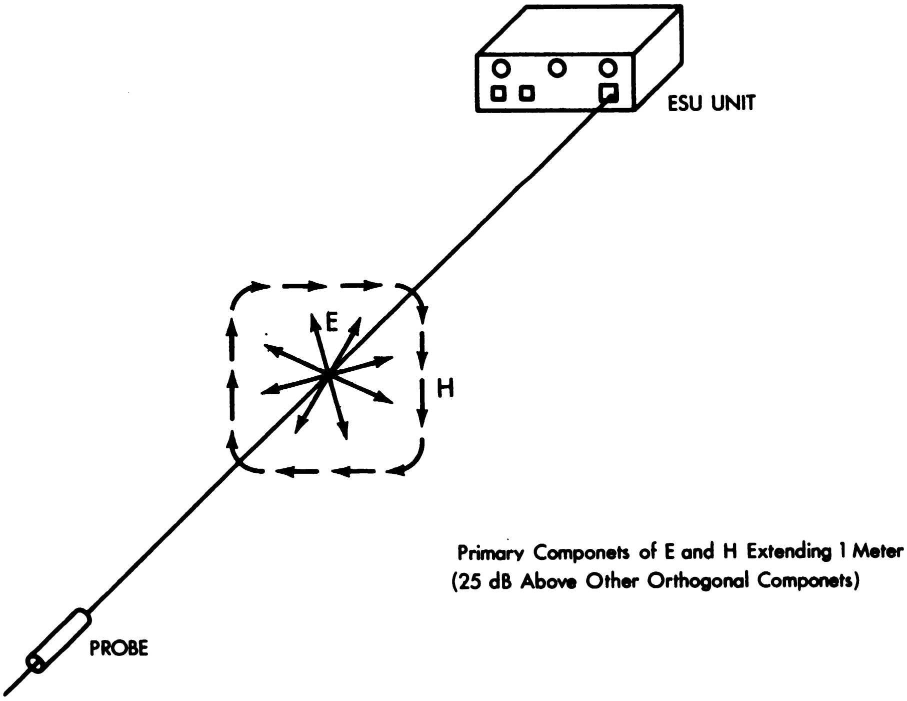
ESU UNIT
E
H
Primary Componets of E and H Extending 1 Meter
(25 dB Above Other Orthogonal Componets)
PROBE

Figure 8. E and H field orientation.

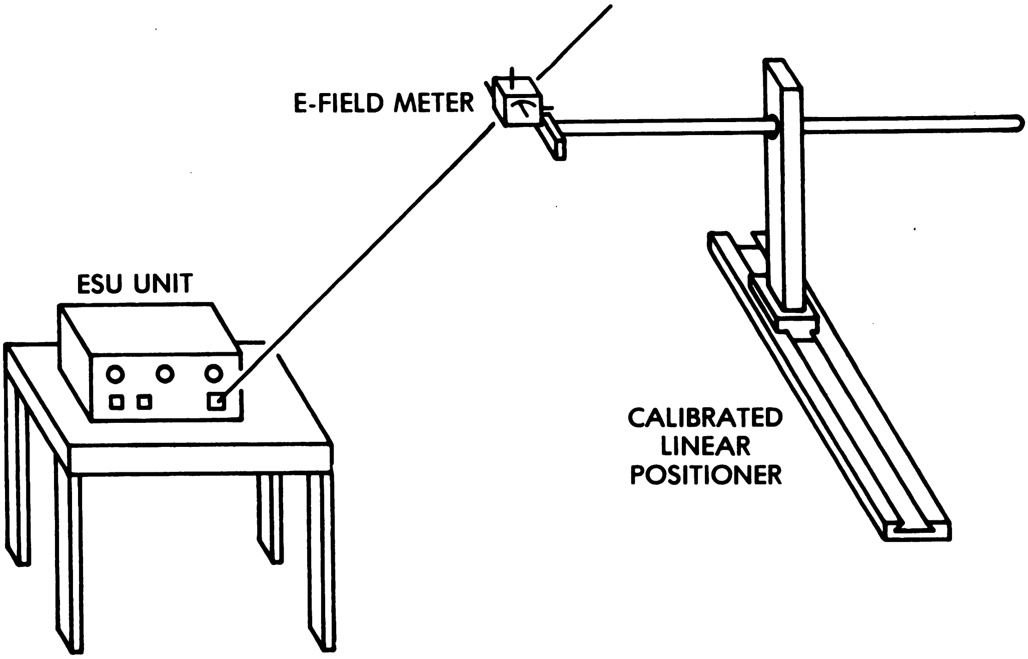
E-FIELD METER
ESU UNIT
CALIBRATED
LINEAR
POSITIONER

Figure 9. Calibrated positioning unit.

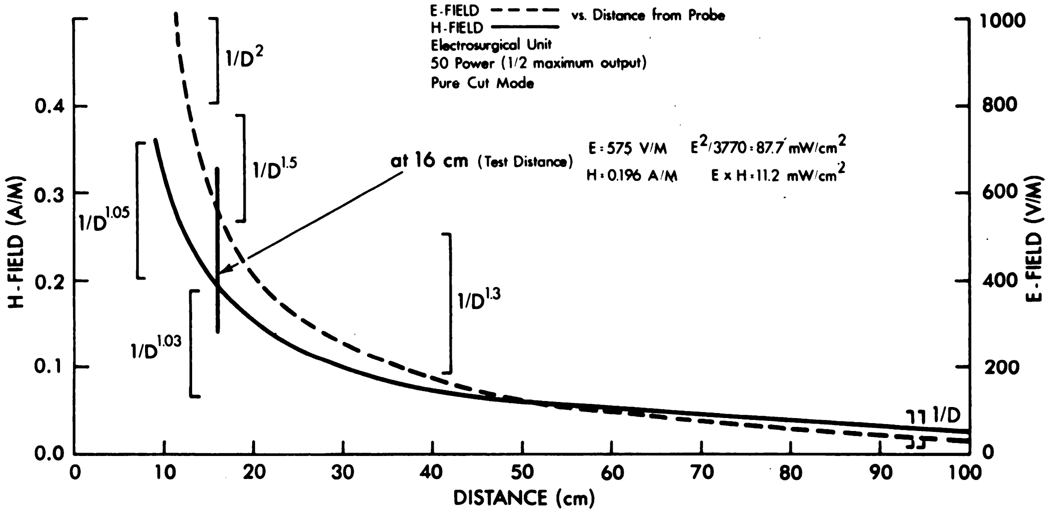

Figure 10. Continuum plot about experimental test distance.

line at 16 cm, the E and H field values under conditions of pure cut, 50 percent power, are very close to those measured on August 18 (in Table 2) at that same distance. The smoothness of the E and H curves serve to substantiate that there are no standing waves or local anomalies present.

Table 2. Solid state electrosurgical unit emissions at 16 cm from probe lead

	E (V/m)	$E^2/3770$ (mW/cm^2)	H (A/M)	E x H (mW/cm^2)
Cutting mode: 50% power				
Open circuit	605.0	97.1	0.22	13.3
Normal cutting	210.0	11.7	0.6	12.6
Arcing	350.0	32.5	0.22	7.7
Coagulation Mode: 30% Power				
Open circuit	620.0	102.0	0.06	4.1
Normal Coagulation	250.0	16.6	0.155	3.8
Arcing	300.0	23.9	0.12	3.6
Hemostasis: 50% Power				
Open circuit	680.0	123.0	0.28	9.0
Normal cutting	270.0	19.3	0.4	10.8
Arcing	350.0	32.5	0.27	9.4
Maximum Hemostasis: 100% Power				
Open circuit	1000.0	265.0	0.71	71.0
Normal cutting	250.0	16.6	0.46	11.5
Arcing	350.0	32.5	0.3	10.5

Table 3. Spark-gap electrosurgical unit emissions at 16 cm from probe lead

	E (V/M)	$E^2/3770$ (mW/cm)	H (A/M)	E x H (mW/cm^2)
Cutting mode: 50% power				
Open circuit	300.0	23.9	0.098	2.94
Normal cutting	250.0	16.6	0.56	14.0
Arcing	260.0	17.9	0.165	4.3
Coagulation mode: 30% power				
Open circuit	300.0	23.9	0.41	12.3
Normal coagulation	140.0	5.2	0.1	1.4
Arcing	120.0	3.8	0.08	1.0
Position 2 Hemostasis: 50% power				
Open circuit	690.0	126.0	0.43	29.6
Normal cutting	300.0	23.9	0.56	16.8
Arcing	300.0	23.9	0.58	17.4
Position 4 Hemostasis: 100% power				
Open circuit	630.0	105.3	0.385	24.2
Normal cutting	200.0	10.6	0.395	7.9
Arcing	600.0	95.5	0.4	24.0

Citizen Band Radio

The most widely used source of EM or RF radiation emissions, and possibly the one with the greatest potential for public exposure, is the citizen band radio (CB) operating at 27 MHz. There are presently 6 million licenses issued by the FCC, each permitting 5 units per license. Other communication units are in operation in specified bands ranging from 3 MHz to 900 MHz. Electric fields 12 cm from a unit hand held (Fig. 11) with output power of 2.5 watts were measured to be as much as 205 volts/meter. This would be equivalent to a free space power density of 11.1 mW/cm^2. At full legal output power of 4 watts, this would be 18.2 mW/cm^2. In using hand-held, portable units, the distance from the antenna to the operator's eye can be as close as 5 cm. (Since these measurements were taken in the near-field the exact radiation fall-off distance was not determined; however, assuming that an inverse square power density relationship exists, this becomes 100 mW/cm^2). The magnetic field at 2.5 watts transmitted power is also given along with its power density equivalent.

A point of major concern in CB is the widespread, though illegal (in terms of FCC regulations), use of linear amplifiers to achieve an output power of up to 1000 watts. Units that also should be considered are those legally licensed mobile units that operate with high output power at other frequencies (police, fire, emergency, etc.). These devices have the potential of exposing people to hundreds of mW/cm^2 at ultra-high and microwave frequencies. Further investigation is planned by BRH in the near future, especially on hand-held units.

CONCLUSIONS

Electric and magnetic field data (along with their equivalent far field power density) have been presented for three electronic products which operate in the radio frequency portion of the electromagnetic spectrum. The data show that in all three cases typical operating conditions can be found in which the currently held though voluntary American National Standards Institute (ANSI) standard of 10 mW/cm^2 is exceeded. The fact remains, however, that very little biological evidence exists concerning the actual danger, if any, to exposure at radio frequencies. It has been demonstrated that it is certainly necessary to measure both the electric and the magnetic field if one is to begin to assess potential hazards--a conventional power density measurement gives quite different results.

REFERENCE

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OPEN DISCUSSION

DR. GUY: I think what you have pointed out is a real problem that exists today in interpreting what you measure around the source. When you talk about near zone fields like this, measuring E and H fields, you also have to talk about the source configuration in the person that might be exposed to this, whether it is partial body exposure or full body exposure. For instance, for a thousand volts per meter, if this was a whole body exposure or whole object exposure situation, you could produce 25 milliwats per kilogram in a perfect sphere; 5 watts per

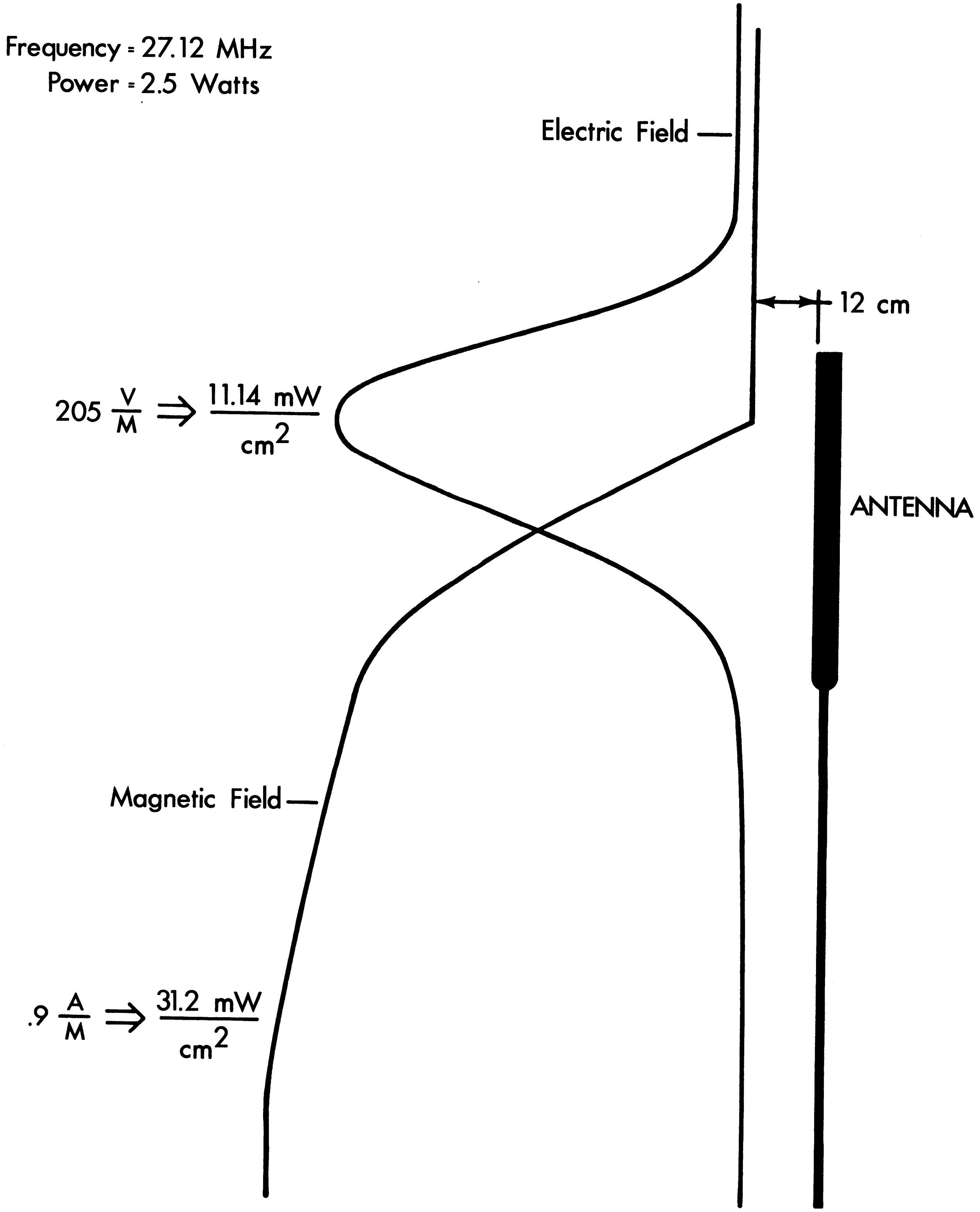
Frequency = 27.12 MHz
Power = 2.5 Watts
Electric Field
12 cm
ANTENNA
205 V/M ⇒ 11.14 mW/cm²
Magnetic Field
.9 A/M ⇒ 31.2 mW/cm²

Figure 11. E and H fields from CB radio antenna at 12 cm.

kilogram in a prolate spheroid stretch about 7 to 1; and for a man model, based on the studies that we have done at 30 megahertz, in the ankle you could produce 80 watts per kilogram. But this would be a result of a free field which is considerably different than the near zone fields from these capacitor applicators, coils, and so forth.

One can get into trouble both ways; one can either be too conservative if one goes one way, or one can be in serious trouble, which I think is the case of a standard now being used in Poland. This is very interesting. We are used to these European standards being conservative; but they have a standard that relaxes as frequency drops and also with the ratio of exposure. It allows magnetic field strengths to get up to the level of 500 Amps per meter, which is probably not even a thermal insult for the particular application that they are talking about. They are talking, probably, about a coil type applicator--very small in volume which would never produce whole body exposure. Even if one were working at the labs at these magnetic field levels, if one uses small animals, one may falsely interpret the situation that there is no hazard. In this kind of a situation, the size of the body is very important--how much tissue and cross-sectional area this magnetic field intercepts. I, therefore, have a feeling that possibly the Polish standard may be based on experiments with small animals without it being properly extrapolated to man; because if that standard were ever applied to some kind of a source that could produce whole body radiation; it would be fatal.

These are the things that we really have to keep in mind, and this is where the importance of the modeling, the SAR, and the dosimetry really comes in; and before any standards can be set in this area, this whole situation should be studied very carefully.

MR. RUGGERA: I agree very much with your comments. I wish that, if anything, I would stimulate some biological research in this area; because I would like to know what is going on.

DR. MUMFORD: You mentioned something about a spark gap generator used by the surgeons and I am not familiar with this equipment, I plead ignorance. Could you tell me something about it? For example, what we used to call decrement, in other words what is the bandwidth of the radiation, could you tell me this?

MR. RUGGERA: I can tell you the principle frequency of the Bovie Unit, and that is what it is normally called, Bovie. Bovie is the trade name that is used by the medical professions that anybody could tell you about, and it is roughly 1 Megahertz. One half to 2 1/2 MHz is the range of the units that are made. But let us say that it is centered at 1 MHz; the frequency spectrum that the spark gap unit puts out is up to 100 MHz. I have measured that and have data on that. I can show you that if you want to come over to the lab.

DR. MUMFORD: Do you mean that it goes from 1 MHz to 100 MHz?

MR. RUGGERA: That is right.

DR. MUMFORD: This is in normal operation?

MR. RUGGERA: Yes.

DR. MUMFORD: A very short, quickly rising pulse then no doubt?

MR. RUGGERA: Yes. They used it for cutting, but they normally use it for coagulation. You use about a 10 kilohertz modulation. With a damped sine wave on a 1 MHz.

DR. MUMFORD: I see. And how did the bandwidth of the measuring instrument compare with the bandwidth of the radiation and the sum of those curves that you measured?

MR. RUGGERA: It turns out that the instrument that I used can measure both. It is a total field type; it is a very broadband instrument.

ELECTRIC FIELD MEASUREMENTS WITHIN BIOLOGICAL MEDIA

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ABSTRACT

Theoretical and experimental analysis on the use of a miniature isotropic electric field probe developed by BRH for implantation electric field measurements within biological tissues is discussed. Analysis employing transmission line model of buried antenna as well as insulated dipoles was performed over the 0.9 to 10 GHz range. The antenna impedance modification as a result of interaction with the surrounding media was computed for a wide range of dielectric parameters typical of various biological materials, over a range of ambient temperatures. It was demonstrated analytically and experimentally that for deep implantation in muscle, relatively little change in probe response occurs with respect to the free space response of the probe over the frequency range of 0.915 GHz to 2.45 GHz. In addition, when in very close proximity to the muscle-fat or the worst case, muscle-air boundary, no significant change occurs in probe response because of dipole impedance variations. Calibration for electric field detection within muscular tissues has also been performed.

INTRODUCTION

Electrical field probe for the measurement of fields within biological media have been proposed and discussed (1-3). No practical probe is available, because in order for a probe to be suitable for implantation measurement, it has to satisfy the following requirements:

1. The probe has to be physically small and biologically compatible.
2. The probe should not perturb the electromagnetic field significantly.
3. The response of the probe should be independent of its surrounding medium.
4. The probe should have isotropic detection characteristics.
5. The probe should be insensitive to temperature variation over a wide range (20° C < T < 50° C).
6. The presence of junction and boundaries should create a minimal perturbation to the probe response.

Bassen and coworkers at the U.S. Bureau of Radiological Health (BRH) have developed a miniature 3-dimensional electric field probe that has been demonstrated to have characteristics that satisfy some but not all of the above requirements (4). The purpose of this paper is to describe the theoretical and experimental development leading to successful modification and calibration of the BRH probe for implantation electric field measurement.

The probe consists of an array of three orthogonal, electrically short dipoles (2.5 mm in length) as shown in figure 1, each coated with a thin layer of epoxy. Each dipole contains a micro-miniature, beam lead, diode detector chip placed directly across the center gap of the antenna. High resistance, thin film leads allow the detected voltage to be monitored under study as a result of microwave current flow on these leads (figure 2). The detector is unique in several aspects. Being a beam-lead chip, it requires no inductive bonding wires for connection to the dipole antenna. This becomes very important at high frequencies, where a resonance of the diode and dipole reactances would seriously degrade the performance of the implanted probe. Secondly, this device utilizes a zero bias Schottky diode, providing very high sensitivity while eliminating the need for either DC biasing of the diode or the use of very high impedance (> 100 Megohm) readout amplifier. The detector diode impedance at microwave frequencies is, however, very high with respect to the antenna impedance. The spatial resolution of this electrically small probe is also quite adequate even when measuring complex fields with steep gradients and short wavelengths within biological material.

There are several important factors one must consider before attempting to calibrate this type of probe for electric field measurement within biological tissues. Upon implantation, the dipole antenna element is submerged in a medium with electromagnetic properties significantly different from those of free space. This may result in a modification of the dipole/diode detection characteristics. Secondly, local inhomogeneities within a single type of biological material, as well as variations in the dielectric properties of a multilayered biological body, may introduce severe limitations in a systematic calibration. Thirdly, a change in the biological body's temperature can alter the dielectric properties of the media surrounding the antennas, resulting in a continuously changing electromagnetic environment. Finally, the problem of the mutual interaction between the antenna and boundaries of discontinuities formed by the interface between various layers of biological materials as well as the surrounding air (such as the muscle/fat/air or muscle/bone interface) must also be analyzed. Mutual interaction between each dipole element is eliminated by the orthogonal, colinear array arrangement. It is important to establish the validity of electric field measurements performed close to boundaries. Boundary interaction problems also arise when external fields are measured in the close proximity of biological specimens. Ideally, a probe for implanted measurements should have a response to the local electric field (or square of the field) so that its detected terminal voltage V_D, as measured across the high resistance detection leads is as follows:

$$V_D = C|\overline{E}|^2 \qquad (1)$$

where V_D = the square-law detected output voltage from the probe, E = local electric field, and C is constant. The constant C should ideally be independent of the constituent electromagnetic parameters of the surrounding medium. A probe with this kind of response not only will eliminate the need for a special calibration of the probe for use in a specific media, but also eliminates problems caused by temperature variations and tissue inhomogeneity within the particular media, once it is calibrated in free space.

THEORETICAL ANALYSIS

Dipole Implanted Within Biological Medium

A well-known theorem in antenna modeling (5) states that the impedance of a short, open antenna normalized to that of its surrounding medium takes on the same value at frequency ω in a medium of index n as it does at a frequency $n\omega$ in

ORTHOGONAL DETECTOR ARRAY

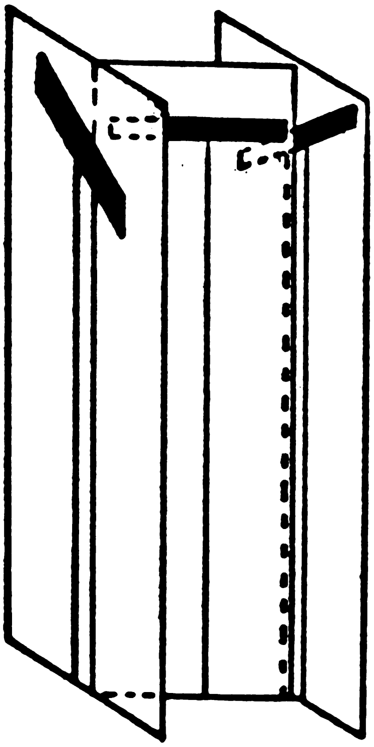

Figure 1. Array of orthogonal dipole with colinear center.

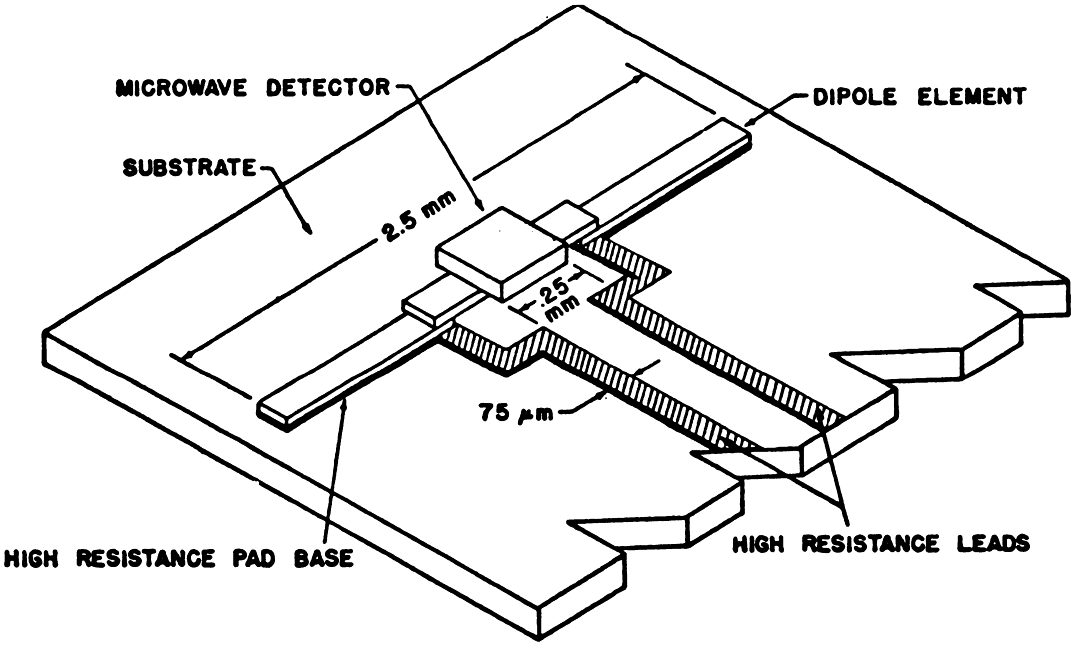
MICROWAVE DETECTOR
DIPOLE ELEMENT
SUBSTRATE
2.5 mm
.25 mm
75 μm
HIGH RESISTANCE PAD BASE
HIGH RESISTANCE LEADS

Figure 2. Miniature dipole-diode detector system.

a medium of index one. This theorem can be expressed mathematically as:

$$\frac{z(\omega,\epsilon^*,\mu)}{\eta} = \frac{z(n\omega,\epsilon_o,\mu_o)}{\eta_o} \qquad (2)$$

where

ϵ^* = the complex permittivity of the medium
μ = permeability of the medium
$\eta = \sqrt{\frac{\mu}{\epsilon^*}}$ - intrinsic impedance of the medium
ϵ_o = permittivity of free space
μ_o = permeability of free space
$\eta_o = \sqrt{\frac{\mu_o}{\epsilon_o}}$ = intrinsic impedances of free space
$n = \sqrt{\frac{\mu\epsilon^*}{\mu_o\epsilon_o}}$ - relative refractive index of medium to free space

It is well known that the impedance of a short, open antenna can be expressed as:

$$z = A\omega^2 + \frac{1}{j\omega c} \qquad (3)$$

where A and C are constants determined by the antenna geometry. If the antenna is much shorter than the wavelength, the constant A can be neglected and the impedance of a short dipole is capacitive as given by:

$$z_d = \frac{1}{j\omega C_d} \qquad (4)$$

where C_d = capacitance of dipole. However, upon implantation in a biological medium, a short dipole is no longer capacitive as indicated by applying equation (2) to equation (4) to give

$$\begin{aligned} z_{d_m} &= \frac{1}{j\omega C\epsilon^*} \\ &= \frac{1}{C_d(\sigma+j\omega\epsilon')} \qquad (5) \\ &= R_m + \frac{1}{j\omega C_m} \end{aligned}$$

where $\varepsilon^* = \varepsilon'(1 - \sigma/j\omega\varepsilon')$ is the complex permittivity of the biological medium.

Note that not only the dipole is no longer purely capacitive, but the impedance is a function of the electrical properties of biological medium (σ,ε'). However by insulating the bare probe with a lossless dielectric, we can reduce the medium dependency of the antenna impedance. The next section will discuss the response of an insulated dipole.

Insulated Short Dipole in Biological Medium

For convenience, the dipole admittance parameter is used instead of the impedance parameter. Consider that an insulated cylindrical probe is formed by adding a concentric dielectric sheath of radius b and length h over a wire of length h' and radius a, and the sheath is assumed to be lossless so that its electrical constitutive parameters are given by ε_i, $\sigma = o$ and μ_o. The analysis is parallel to that of Smith and King (6). The admittance for the bare probe in the medium and the capacitance of the insulating sheath are given by

$$\begin{aligned} Y_m &= C_d(\sigma + j\omega\epsilon') = G_m + j\omega C_m \\ Y_s &= j\omega C_s \end{aligned} \qquad (6)$$

where C_d and C_s are capacitance of the bare in free space and of the insulation sheath as given by Smith (7).

Figure 3 shows the circuit representation for the input admittance of the bare and insulated electric field probes. The admittance of the insulation capacitance in series with the bare probe yields an expression for the input admittance of an insulated probe in biological medium to be

$$Y_i = C_d\left\{\sigma\left[\frac{\gamma^2}{p^2+(1+\gamma^2)}\right] + j\omega\epsilon'_i\left[\frac{p^2+(1+\gamma)}{p^2+(1+\gamma)^2}\right]\right\} \qquad (7)$$

where

$$\gamma = \frac{\epsilon'_i}{\epsilon'}\,\frac{\log(h/a) - 1}{\log(b/a)} \qquad p = \tan\delta$$

and a and b are the radius of the antenna and insulation respectively.

The design objective is to formulate a combination of antenna geometry such that Y_i is dominantly capacitive and and independent of the biological medium.

Let us first consider the case of the BRH probe. ($a = 0.1$ mm, $b > 2a$, $h = 2.5$ mm). For the range of $o < \tan\delta < 0.5$ and $5 < \varepsilon' < 50$, the ratio of G_i and ωC_i is given by (7):

$$\frac{G_i}{\omega C_i} = \frac{P\gamma}{P^2+(1+\gamma)} \qquad (8)$$

It can be shown that for the BRH probe operating at 1 - 10 GHz in materials with the above range of electrical parameters, G_i can be neglected so that Y_i is predominantly capacitive and is given by

$$Y_i = j\, C_d\epsilon_i\, G(P,\gamma) \qquad (9)$$

where $G(P,\gamma) = P^2 + (1+\gamma) / P^2 + (1+\gamma)^2$, is dependent on probe geometry, insulation geometry and electrical properties of the surrounding medium.

One primary requirement for a probe to be suitable for implantation measurement is the G varies insignificantly with changes from material to material. By proper antenna design, such criterion can be met. Figure 4 shows the variation G as a function of the ratio $\varepsilon_i/\varepsilon'$ for various insulation thickness ($b = 2a$, $3a$ and $4a$) for the BRH probe in a material with $\tan\delta = 0.2$. For $\varepsilon_i = 2$, the graph covers ε' from 5 - 50 which is typical of biological tissues. Figure 4 shows that for this particular case, an insulation thickness of $b \simeq 3.5a$ is the optimum design for a material independent probe. Figure 5 shows the variation of G as a function of $\varepsilon_i/\varepsilon'$ for $\tan\delta = 0.1$, 0.3 and 0.5 for the BRH probe when $b = 2a$. If we limit our range to $15 < \varepsilon' < 50$ (typical of muscular tissues), then the error introduced caused by changes in ε' will be always less than 10 percent. Note that if the insulation thickness is increased, our detection range capability for different ε' will be expanded as evidenced from the previous figure. Figure 6 shows a plot of variations in G as a function of ε' and ε_i for $\tan\delta = 0.2$, $a = 0.25$ mm, $b = 0.5$ mm and $h = 2.5$ mm (which is the initial design of the BRH probe). The plateau-like dependence of

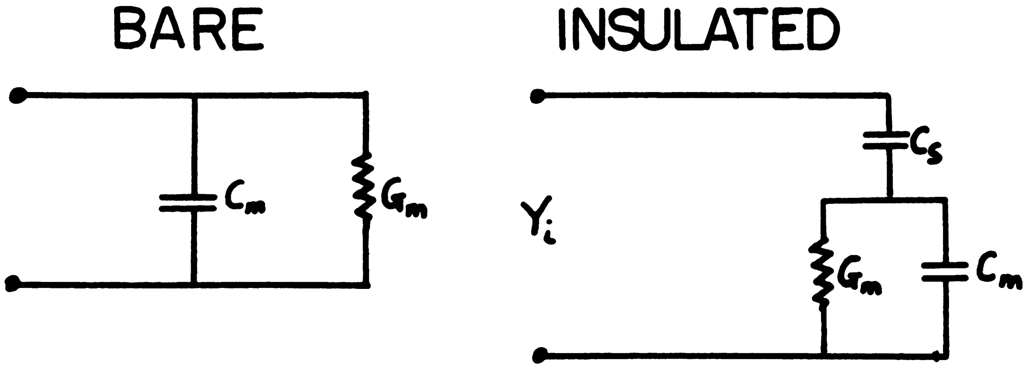

Figure 3. Input admittance circuit representation of bare dipole and insulated dipole in material media.

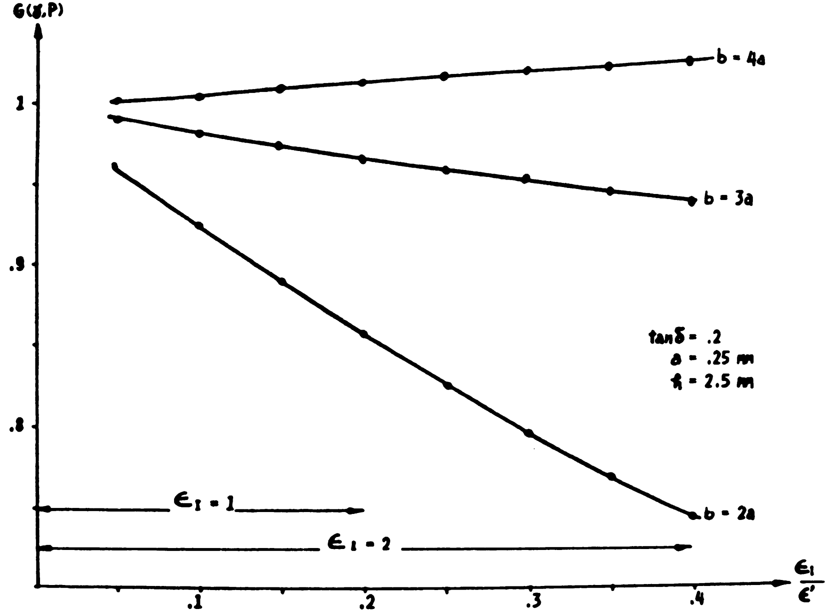

Figure 4. The variation of G(γ,P) as a function of ϵ_I/ϵ' for various insulation thickness. Note that the full range of ϵ_I/ϵ' needed to cover the values of $5 < \epsilon' < 50$ depends on the value of ϵ_I used. In this case, the values of tanδ, a and h' are .2, .25 mm, and 2.5 mm respectively.

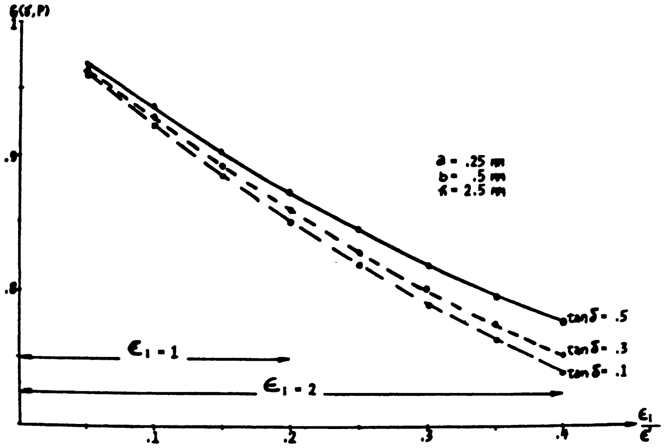

Figure 5. Variations of G(γ,P) as a function of ϵ_I/ϵ' for various values of loss tangent for a = .25 mm, b = .5 mm, and h' = 2.5 mm.

G for $\varepsilon' > 20$ suggests that for implantation measurements in muscular tissues ($\varepsilon' > 20$), the BRH probe is material independent.

Note that the above insulated dipole analysis is valid only for frequency range over which the dipole is short compared to the wavelength. In biological tissues, the wavelength is significantly shortened by a factor directly proportional to the square root of the dielectric constant which suggests, at a first glance, that 9 GHz ($\lambda_d \simeq .5$ cm) is the upper limit of the frequency capability of the BRH probe. Yet, the presence of insulation can reduce the wavelength shortening. Thus, in order to analyze the BRH probe response over a wider frequency range (0.5 GHz $< f <$ 10 GHz), the theory of buried transmission line is used (8).

Theory of Buried Transmission Line

For insulated antenna buried in a conducting medium, the transmission line theory can be used to obtain estimates of current distribution in the antenna and of the antenna impedance. The case we consider is an insulated dipole buried at a depth h inside an infinite planar slab of muscular tissue bound on the outside by a fat layer as shown in figure 7. The insulated dipole can be modeled as an open-ended single wire transmission line of radius "a" coated with epoxy insulation of thickness (b-a). The wire is immersed in a lossy dielectric with dielectric constant $\varepsilon^* = \varepsilon' - j\varepsilon'$, similar to that of muscular tissues. The line is excited by a current $I = I_0 e^{-\tau z}$ along its axis and the resultant field is a superposition of a primary field and a secondary reflection as a result of the boundary discontinuities between muscle and fat. This field is calculated with respect to matched boundary conditions so that a dispersion relation results. From the dispersion relation, one obtains τ, which is the propagation constant along the wire. This constant is calculated as (9):

$$\Gamma = i\, k_o \sqrt{\epsilon_i'} \left[1 - \frac{1}{\log(b/a)} \left(\frac{-\, H_o^{(1)}(k_o\sqrt{\epsilon^*}b)\; f(b,o,h)}{k_o\sqrt{\epsilon^*}b H_1^{(1)}(k_o\sqrt{\epsilon^*}b)} \right)\right]^{1/2} \qquad (10)$$

where

ε_r = relative dielectric constant of the muscle

k_0 = free space wave number

$H_0{}^{(1)}$ and $H_1{}^{(1)}$ are Hankel Functions

and f(b,o,h) is a multiplying factor dependent on the properties of the boundary discontinuity and the burial depth "h."

Equation 10 was derived by assuming that the conductivity of the wire is infinite. Guy has derived an expression for antenna resistance (4) and reactance (X_A) using the relationship, $\tau = \alpha - j\beta$ as follows (9):

$$R_A = \frac{120}{k_o\epsilon_i{}'} \log\left(\frac{b}{a}\right)\left(\frac{-\beta\sinh\alpha\ell - \alpha\sin\beta\ell}{\cosh\alpha\ell - \cos\beta\ell}\right)$$

$$X_A = \frac{120}{k_o\epsilon_i{}'} \log\left(\frac{b}{a}\right)\left(\frac{\beta\sin\beta\ell - \alpha\sinh\alpha\ell}{\cosh\alpha\ell - \cos\beta\ell}\right) \qquad (11)$$

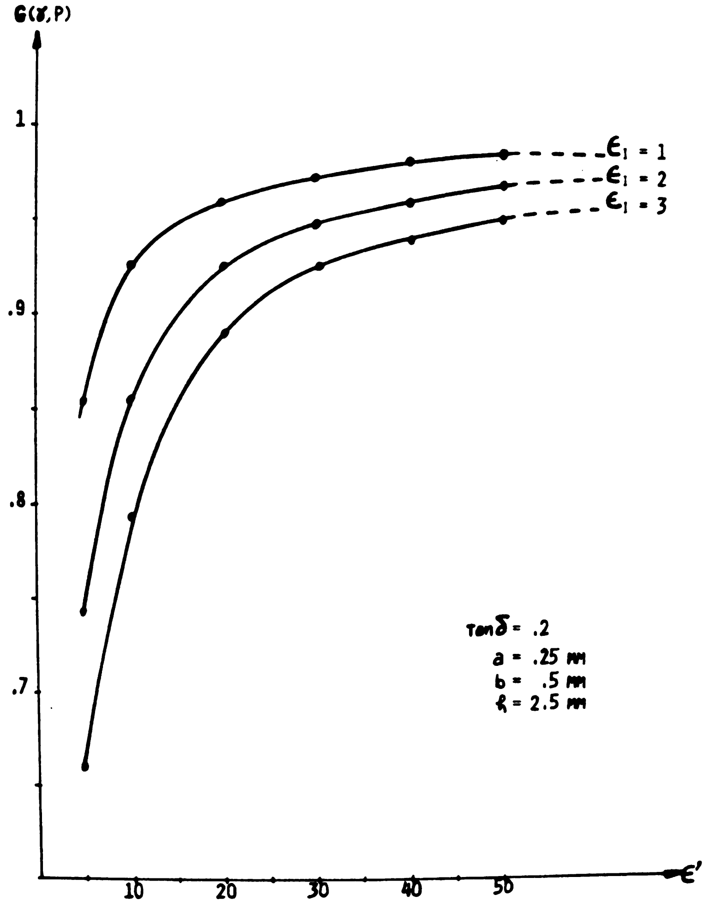

Figure 6. Variation of G(γ,P) as a function of ε' for various insulation dielectric properties. Note that as ε_I increases, its shielding effectiveness decreases.

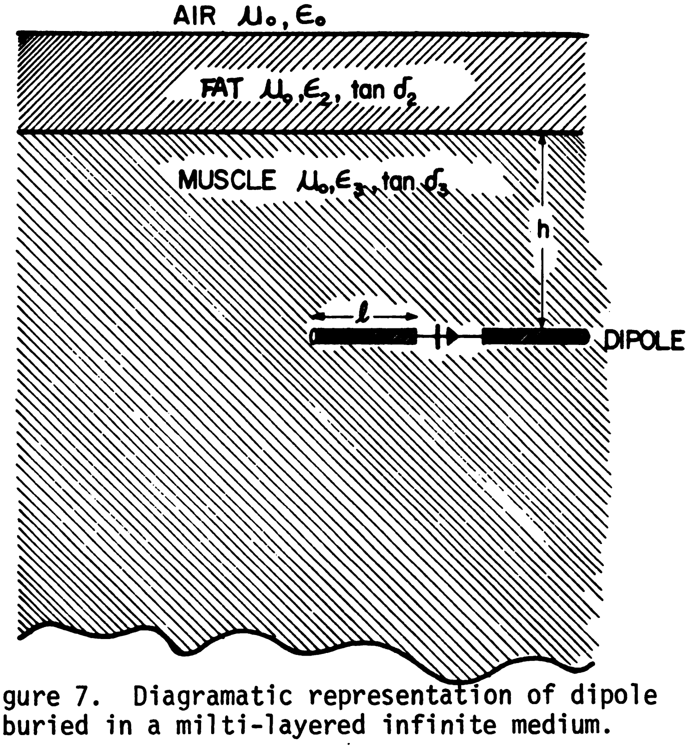
AIR μ_o, ϵ_o
FAT μ_o, ϵ_2, tan δ_2
MUSCLE μ_o, ϵ_3, tan δ_3
h
ℓ
DIPOLE

Figure 7. Diagramatic representation of dipole buried in a milti-layered infinite medium.

For the initial calculation, the burial depth is assumed to be infinite, so that $f(b,o,h) \rightarrow 1$. We generate values of R_A and X_A, at frequencies ranging from 1 - 10 GHz, with the BRH probe dipole geometry for values of ε' ranging from 35-45 and $\tan\delta$ from 0.1-0.4 is shown in figures 8 and 9. During irradiation, temperature elevations may occur under high exposure situations. The change in the dielectric properties of simulated muscle has been experimentally measured at frequencies in the vicinity of 8.5 GHz over a range of 20° C to 50° C (figure 10). Very similar variations occur in the 1-3 GHz range. The findings suggest that these changes in the dielectric properties of biological materials must be considered when the impedance of an implanted antenna is computed. However, note that the range of ε' and $\tan\delta$ considered in figure 8 overlaps with that because of temperature variation from 20° C - 50° C. Thus, the response of the antenna also can be considered to be temperature independent for $35 < \varepsilon' < 45$, 0.1 $\tan\delta < 0.4$ and $20° \text{ C} < T < 50° \text{ C}$, as evidenced by figures 8 and 9.

BRH Probe Response

Let us now consider the practical detection characteristics of the BRH probe, figure 11 shows the equivalent circuit representation of the antenna-diode detection junction. The following equations govern the detection characteristics of the BRH probe:

$$\begin{aligned}
V_D &= -\,2h\,|\bar{E}|\,(Y_i/Y_i+Y_\ell) = K|\bar{E}| \\
V_{D_x} &= K_x \sin\theta \cos\phi\ |\bar{E}| \\
V_{D_y} &= K_y \sin\theta \sin\phi\ |\bar{E}| \\
V_{D_z} &= K_z \cos\theta\ |\bar{E}| \\
V_D^2 &= V_{Dx}^2 + V_{Dy}^2 + V_{Dz}^2
\end{aligned} \qquad (12)$$

where

V_D = voltage across diode on each antenna

$|E|$ = electric field strength along the antenna

V_D = square of the sum of the detected voltage

and K_x, K_y and K_z are detection constant of the individual orthogonal antenna element.

In practice, K_x, K_y and K_z can be slightly different. However, the amplified detected voltage can be compensated for by adjusting the gain of the external amplifier so that the effective gain of each channel is identical. Under such conditions and if the diode is biased at the square law detection region, then the detected voltage measured at the output of the amplifier is given by:

$$V_P \propto V_D^2 = K^2|\bar{E}|^2 \qquad (13)$$

Satisfying equation (13) will guarantee isotropic detection characteristics of the probe. However K^2 in equation (13) is a function of Y_i and Y_ℓ that are in turn functions of frequency and dielectric properties. An ideal probe should have K^2 independent of both change in frequency and change in electrical properties of the medium. We have demonstrated that for the BRH probe, Y_ℓ is relatively insensitive to change in ε' and $\tan\delta$ for $\varepsilon' > 20$ over the frequency

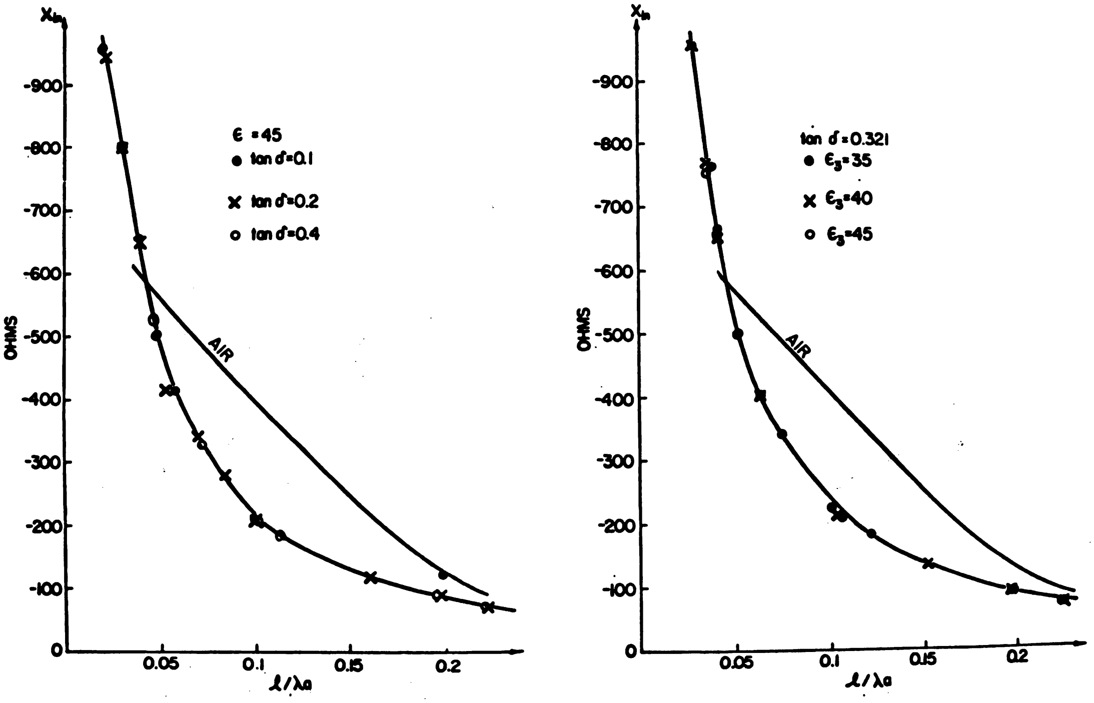

Figure 8. Antenna reactance for BRH dipole in various media ($.1 < \tan\delta < .4$; $35 < \varepsilon' < 45$) as a function of electrical length. The range of $.05 < \ell /\lambda a \leq .2$ covers 0.9 to 10 GHz.

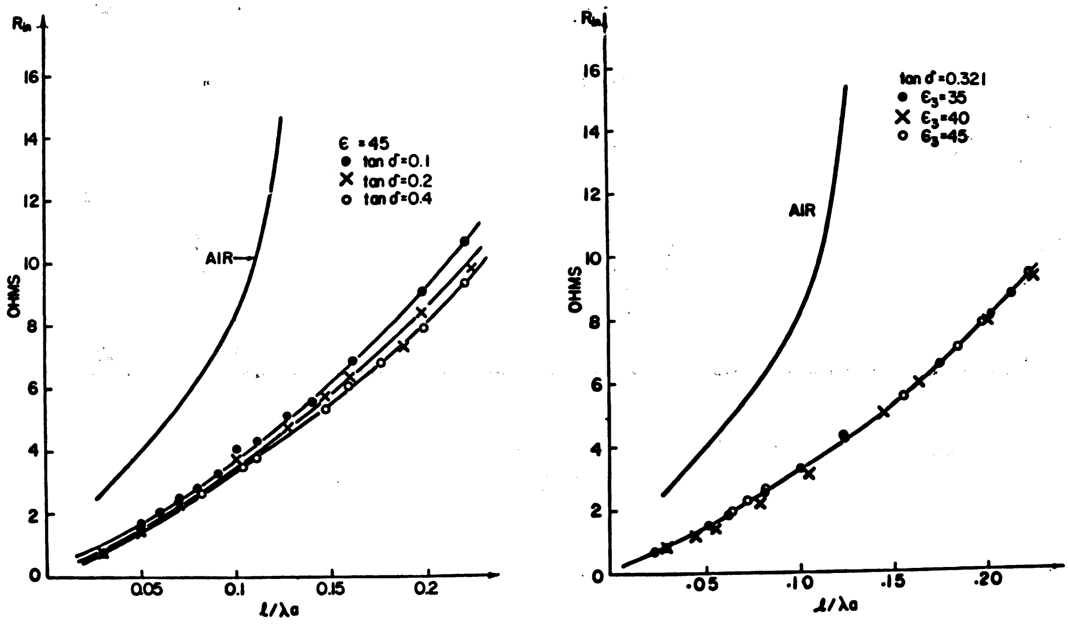

Figure 9. Antenna resistance of BRH dipole in various media ($.1 < \tan\delta < .4$; $35 < \varepsilon' < 45$) as a function of electrial length.

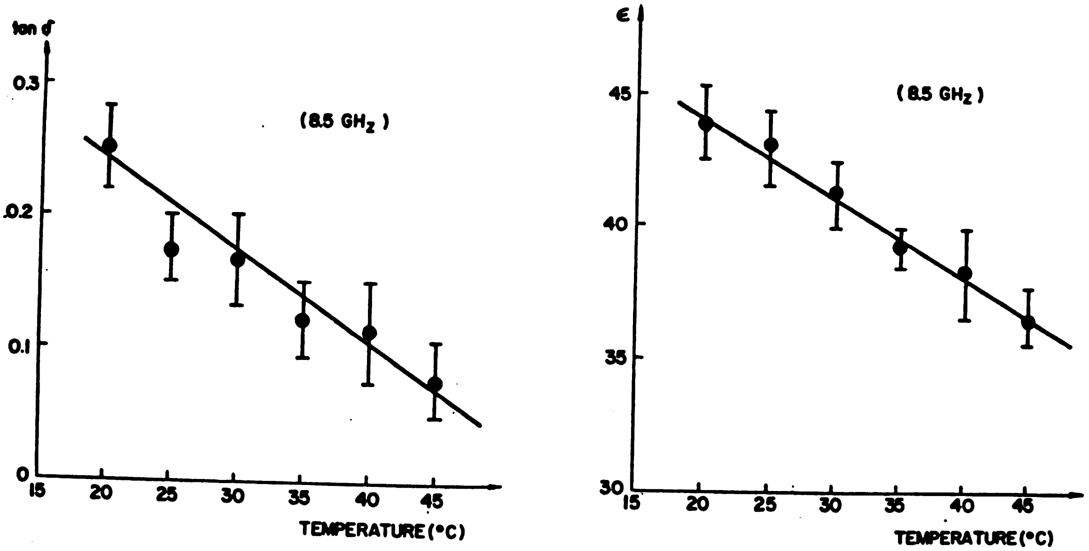

Figure 10. Temperature dependence of ε' and tanδ between the 20° C to 45° C range.

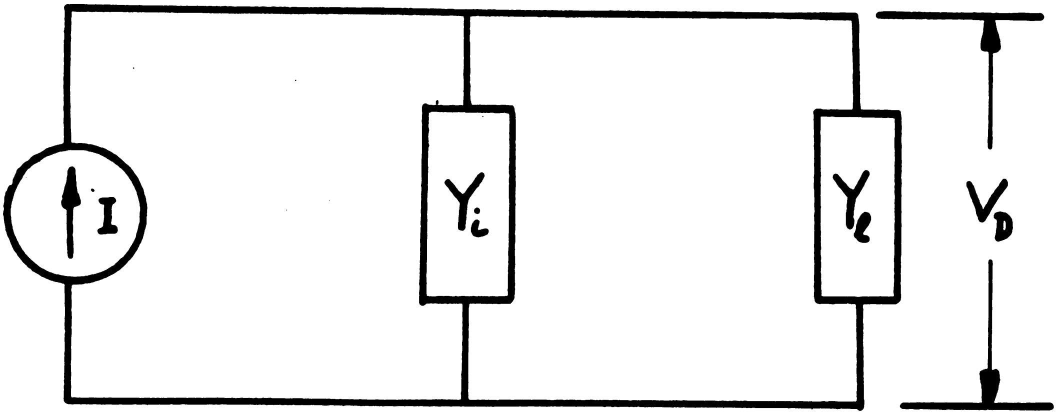

Figure 11. Equivalent circuit representation of the antenna diode-dipole detection junction.

range of 1 - 10 GHz. Let us now examine the frequency dependence of K^2. The nominal capacitance of the Schottky diode given by the manufacturer is on the order of 0.1 picofarad. From results indicated by figure 8, Y_i is approximately 10^{-2} mhos at 10 GHz and 10^{-3} mhos at 1 GHz. In comparison, Y_ℓ is always much smaller than Y_i so that V_D can be written as

$$\begin{aligned} V_D &= -\ 2h'|\bar{E}|(Y_i/Y_i+Y_\ell) \qquad (14) \\ &\simeq\ 2h'|\bar{E}| \end{aligned}$$

Note that h is totally independent of the frequency and electrical characteristics of the surrounding. Thus, when buried in an infinite muscular medium, the BRH probe satisfies conditions 1-5 that suggest that it is suitable for electric field detection within such tissues. However, as indicated by Guru and Chen (3), junction effect can introduce significant modification of the antenna characteristics of a thinly insulated dipole, causing the measured values of electric field to be location dependent. The boundary effect was included in equation (10) by the modification factor f(b,o,h) given by:

$$\begin{aligned} f(b,o,h) = 1 + \frac{1}{\pi H_o^{(1)}(k_o\sqrt{\epsilon^* }b)} \int_{-\infty}^{\infty} & \frac{\sqrt{k_o^2\epsilon^*-\xi^2} - \sqrt{k^2-\xi^2}}{\sqrt{k_o^2\epsilon^*-\xi^2} + \sqrt{k^2-\xi^2}} \\ & * \ \frac{\exp(-j2h\sqrt{k_o^2\epsilon^*-\xi^2})}{\sqrt{k_o^2\epsilon^*-\xi^2}}\, d\xi \qquad (15) \end{aligned}$$

where

$k^2 = k_o\varepsilon_r$

ε_r = permittivity of external medium,

ε^* = permittivity of the tissue

Equation (15) can be analyzed numerically to generate information for a full assessment of planar boundary effects. However, note that the value of f given by the equation can never exceed two such that even under the worse case, (total reflecting boundary) the change in value of Y_i due to boundary interference is never expected to drop below half its original value. Thus, for detection within muscular tissues, the maximum error in V_D as indicated by equation (12) as a result of boundary effect can never exceed 17 percent for the BRH probe. ($Y_i \simeq 10Y_\ell$), and for implantation measurement in lossy medium such as muscular tissues, we expect that error caused by junction should be minimal except very close to the boundary ($h << \lambda_o\ (\varepsilon^*)^{-\frac{1}{2}}$).

EXPERIMENTAL CALIBRATION

The antenna was calibrated within an "infinite" slab of simulated muscular tissue (30 cm x 30 cm x 15 cm) whose dimensions are large with respect to wavelength within the material. This material was formulated as specified by Guy with loss tangent values of 0.5 and 0.35 at the respective frequencies of 0.915 and 2.45 GHz and a dielectric constant of 50 at both frequencies. The antenna was inserted from access openings in the rear of the slab and the whole assembly was situated within an anechoic chamber with the front of slab exposed to a known plane wave at 2.45 GHz and at 0.315 GHz. The incident and scattered fields in front of the slab were measured to allow the net internal field to be analytically computed. The experimental arrangement is shown in figure 12.

This "infinite" slab was placed directly behind a lossless matching plate (one quarter wavelength thick at 2.45 GHz) to minimize scatter of the dielectric body. To ensure that no standing waves existed within the slab in the transverse (X-Y plane) and the longitudinal directions (where the incident field is propagating along the longitudinal "Z" axis) and thereby demonstrating that the slab was "infinite," probe readings were measured along both X and Y axes versus implantation depth. Figure 13 shows the measured probe response for various positions in the X-Y plane at 2450 MHz. Note that the plots of probe voltage outputs along the longitudinal direction in each transverse plane are virtually identical exponential curves which support the assumption that no standing waves exist within the media.

Probe output voltage data was taken at both 2.45 and 0.915 GHz for various incident power density levels within an anechoic chamber. Figure 14 shows the measured probe response versus theoretical values of the field existing within the infinite slab. The calculated theoretical field strength squared ($|E|^2$) is plotted versus depth "h," and is shown as a continuous line along with the data generated from a typical calibration at 2.45 GHz within a muscular phantom. The same type of data was also obtained at 0.915 GHz. A comparison was made between the absolute response of a probe to a given, computed field strength within muscle equivalent material and the probe response obtained in a free-space calibration at the same field strength. It was found that the experimental and theoretical response in muscle tissue agreed within ± 10 percent when the free-space probe sensitivity coefficient was directly used. Thus, it was concluded that once calibrated in free space, this probe can be used for accurate measurements of E-fields within infinite slabs of muscular tissues. The response of the probe can be equated with the actual electric field strength existing in muscular tissue at frequencies ranging from 0.915 GHz to 2.45 by simply using the free-space calibration factor for the probe. Since multilayered, finite biological systems are of primary interest, further experiments were performed as follows to determine boundary effects on the probe calibration.

Experimental Study of Probe Response in the Proximity of Boundaries

In order to study the effects of probe interaction with the multiple layers and their boundaries, that always exist in biological systems, a series of experiments was performed. The results were used in conjunction with the previous theoretical antenna analysis using the buried transmission line model in the proximity of the muscle-fat boundary. It was known that in the proximity of this or any boundary between different dielectric materials (including air) the currents induced in the probe antenna would reradiate microwave energy. When this reradiated energy was reflected from a boundary, back to the antenna, the net current flow on the antenna, and hence its impedance, would change. In very close proximity to the reradiated energy. This proximity effect is accounted for by the parameter f(b,o,h) in equation (15). The increasing proximity to such a boundary would cause an exponentially increasing sinusoidal oscillation in antenna impedance, for probe travel toward the boundary surface, along an axis which was normal to the surface.

The worst case condition of an air-muscle interface was chosen for the experimental evaluation of the probe-boundary interaction. At 0.915 GHz, the plane wave irradiation experiment with an "infinite" slab of muscle material was performed as previously described, except that the matching plate was removed and a low density styrofoam slab was placed on the front surface of the muscle material. Continuous probe scans were made from the rear of the slab to the front of the slab, with the probe body oriented parallel to the axis of propagation. The central probe dipole was oriented perpendicular to the axis of propagation and parallel to the electric field. Using a mechanical positioner, well-covered with microwave absorber, the probe was slowly pushed through the

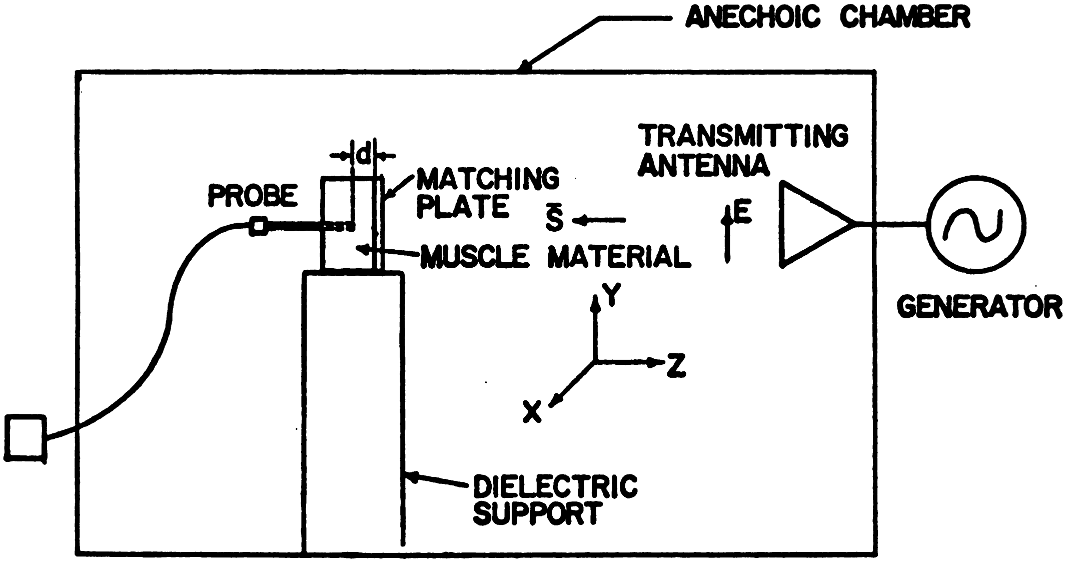
ANECHOIC CHAMBER
TRANSMITTING ANTENNA
d
PROBE
MATCHING PLATE
$\bar{S}$
E
MUSCLE MATERIAL
GENERATOR
Y
Z
X
DIELECTRIC SUPPORT

Figure 12. Experimental arrangement of system for calibration of BRH probe for E-field detection.

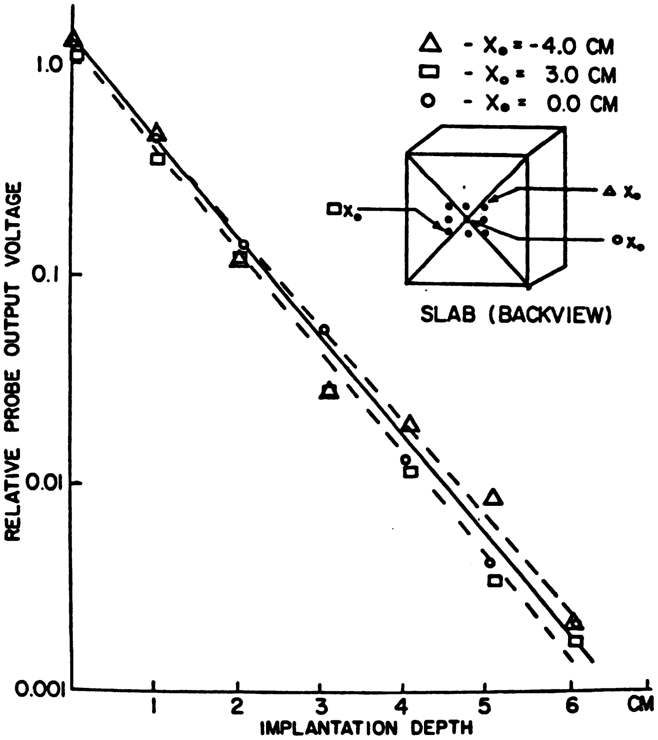

Figure 13. Probe output voltage as a function of depth along asymmetrical paths at 2450 MHz.

entire muscle slab until it emerged in the styrofoam. The probe displaced muscle equivalent material during its travel, leaving a hole approximately 5 mm in diameter when withdrawn. The probe itself was found to be well coated in muscle material when withdrawn, ensuring complete emersion in the muscle-equivalent material during measurement. Similar data was taken at 2.45 GHz, using a near-field irradiator (a horn antenna, very close to the slab's front surface). Data is presented in figure 15 for both frequencies. As is evident, no significant oscillations occur in the probe output voltage as the distance of the probe to the boundary approaches and reaches zero. This qualitative data is conclusive proof that the diode impedance is high with respect to the antenna impedance, since the antenna impedance that is known to oscillate, has no effect on the probe output. Therefore, the probe may be used without concern for boundary effect errors in multilayered biological systems or for shallow implantation in noninfinite biological media. Bone and fat have a lower loss tangent than muscle, and boundary interactions may be slightly more significant for the probe under discussion. Therefore, measurements of the electric field inside complex bodies composed of these materials can be accurately performed; but some further measurement in low-loss material is needed.

Experimental Evaluation of the Perturbation of Fields Existing in Simulated Biological Media by the Implanted Probe

To make certain that the electrically small probe did not perturb the electric fields being measured, and thus did not alter the distribution of energy within the previously unpenetrated system, an experiment was performed at 2.45 GHz. Using a TEM, near-field applicator, the previously described muscle-equivalent slab with styrofoam front plate was irradiated while a probe was placed along the center axis of the slab, parallel to the axis of propagation. The center dipole was placed 2.5 cm from the front surface of the test "phantom." This dipole was aligned with the incident electric field and remained stationary while a second probe was inserted from the top of the slab, with its handle aligned with the electric field. This second probe was continuously pushed toward the first probe until the two tips touched. The output of the stationary probe was continuously monitored. No observable change in probe output was observed until the proximity between tips was less than 1 cm. Then the probe output increased to a value 10 percent higher than previously observed. This test demonstrated negligible perturbation by the probe body and its three orthogonal dipoles for implantation in loss media. Again, further tests in low-level material are needed.

CONCLUSIONS

The use of an electrically small, three axis dipole probe for direct measurement of electric fields at microwave frequencies (0.915 to 2.45 GHz) has been experimentally and theoretically explored with implantation in biological media as the goal. Using a well-established, buried transmission line and insulated dipole theoretical model, the antenna impedance has been analyzed with respect to various implantation situations. The use of a small, insulated dipole as a detection element enables the probe's response to an electric field to remain relatively constant with regard to the electrical properties of the media surrounding the probe. Material inhomogeneities and temperature changes, therefore, do not affect this relationship, even though these produce significant variations occurring in the magnitude of the dielectric constant of the biological media. Therefore, a properly designed probe may be calibrated in free space, and then used as an implantable probe in "infinite" media without spatial calibration. Using this approach, errors of less than ± 10 percent were encountered when fields experimentally measured in a large muscle-equivalent

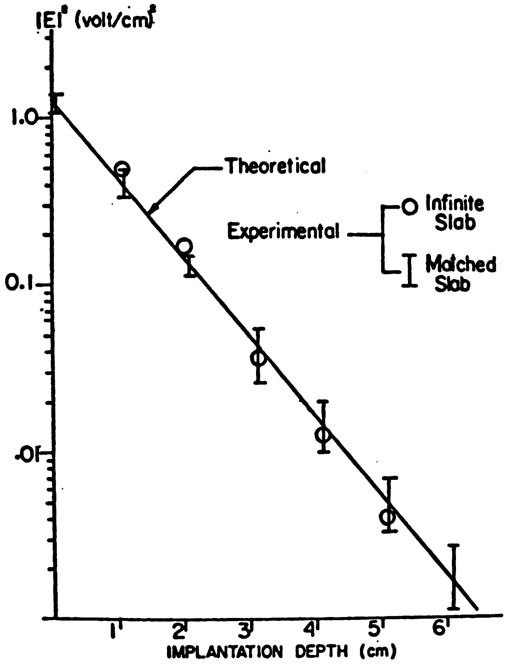

Figure 14. Comparison of absolute values of experimentally measured and predicted electric field strengths in muscle-equivalent slab at 2450 GHz (normalized representation).

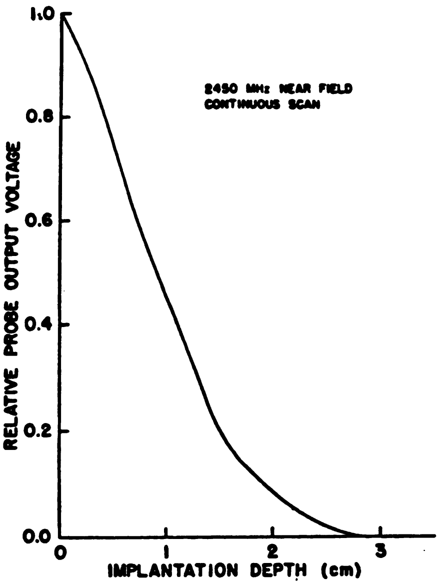

Figure 15a. Experimental probe output from continuous scan towards muscle-air boundary at 2450 MHz.

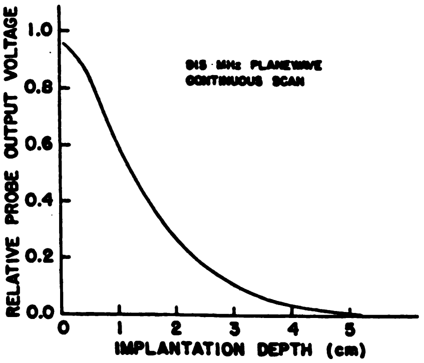

Figure 15b. Experimental probe output from continuous scan towards muscle-air boundary at 915 MHz.

slab were compared with theoretically computed field strength induced by plane wave irradiation of the slab at 0.015 to 2.45 GHz.

The problems associated with measurements performed in biological systems near the boundary layers between various biological materials (muscle-bone, muscle-fat-air) were also considered. Worst case (muscle-air) boundary conditions were experimentally measured with continuous scans along an axis normal to the boundary plane. The experimental findings demonstrated that no detectable probe-boundary interaction occurred during increasing proximity to the boundary. This is unique to the specific probe design utilized in which the diode detector impedance is much higher than the antenna impedance, thus eliminating dipole-boudary interaction as described in the theoretical analysis. The lack of significant electric field perturbation by the probe itself was also demonstrated in the muscle-equivalent slab.

The performance of this probe can be further improved by optimizing the design of the insulation thickness. A detailed numerical analysis of equation (15) for the variation in f (b,o,h) under all implantation conditions (especially in low loss tissues) will give a full assessment of errors due to junction interference.

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OPEN DISCUSSION

DR. RABINOWITZ: How large an area do you integrate over with this probe?

DR. CHEUNG: The probe length is 2.5 millimeters.

DR. RABINOWITZ: No, what I am asking is the special resolution. If the dielectric constant were to change within the system within what you are measuring, how constant does the dielectric constant have to be in spatial terms?

DR. CHEUNG: How constant does the dielectric constant have to be? Can you be more specific?

DR. RABINOWITZ: Yes, I mean it is going to vary in a biological system; it is a function of position in space.

DR. CHEUNG: In space? Maybe Howard Bassen has a better idea; I thought you did something about the spatial resolution tests.

MR. BASSEN: If you look at figure 15, you will see that it goes from ±3 centimeters in a sphere. Therefore, all of those oscillations are over an area of about 1 centimeter period. The point is, that certainly within a centimeter, we reproduce these complex spatial variations very accurately, since the probe is 2.5 millimeters. If the gradients are larger than 2.5 millimeters, then that includes gradients as a result of different dielectric constituents and we can still measure these effects. Also, by being insulated, the probe is made relatively insensitive to the particular dielectric constant, and we eliminate the effects of the inhomogenous material of a true biological system.

DR. GUY: Normally when you are in an inhomogeneous tissue, you do not know in which direction the fields are; they can be in any direction. This is where you have to introduce design B, using three orthogonal probes; but in order to insert this into tissues under practical conditions, it brings up a point that I have argued many times with Ron Bowman about. You have to incapsulate this in some dielectric material. Considering a spherical shape, we have an outside field, E_0 and and inside, field E_I. (You can take different shapes, but there is always some relationship between the field inside and the field outside.) Isn't the field inside for a sphere, related to the dielectric as such $^3\varepsilon_0 + \varepsilon/\varepsilon_0$ on the inside (or I may have inverted it). However, the point that I wanted to make is that if this orthogonal array of probes emerges from this sphere and goes from a muscle medium to a subcutaneous fat medium, there is going to be a definite change in the E field that the probes are measuring inside. How do you eliminate that problem, or is that possible?

DR. CHEUNG: We are not using this configuration right now, as far as the sphere is concerned, and naturally this question will be better answered after we hear Howard Bassen's presentation. I can just briefly say, that this is a strip line geometry. The last version of the probe only consisted of one probe at a certain angle. When you put it in the media and rotate it, you cover all three of the orthogonal directions. The way to insulate the strip line is that your insulation be layered. You can go back to the same one-axis probe again, so you do not have to deal with three orthagonal probes anymore. Howard Bassen will talk about it in more detail.

MR. BASSEN: Again, referring to figure 15, the last curve was taken with a single axis probe with a hemispherical insulator. The sphere, or in this case the hemisphere, is small compared to the wavelength in the media. This internal field effect does not really apply, I do not believe, because we get good agreement between the theoretical fields and the measured field in muscle material and in air. So I think this only holds true when you have an electrically large sphere, and then you have these effects of the field being altered within the media.

As I said, we get very good agreement between theory and measurement even with this hemispherical tip that we placed over the probe, since the dipole is an infinitesimal approximation being 2.5 millimeters. Incidentally, though, we have found in multi-layered spheres, theoretical studies and in biological specimens, and this is only preliminary, that we have not seen polarization rotation from the external plane-wave field in the media. So this can be a problem, but we have not seen it for a plane wave impinging on a purely dielectric body of different properties. We have not seen it in various experimental cases, including animals.

DR. GUY: Yes. You would not see it in a homogeneous sphere, but in animals with curved layered media, I do not see how you could prevent the change of orientation of the fields, especially over a wide frequency range. Evidentally, what is holding here is the probes are probably perturbing the fields so much in this small sphere that it is essentially sensing the outside field, but there must be some transition. If the sphere is made larger, you are going to have to go from this situation into a situation where you do get this true relationship where the probe is no longer perturbing the fields inside this sphere. So, I think there should be some kind of a study made where this transition takes place to find what the limitations are.

DR. CHEUNG: However, it is still true that the one with the single probe by itself will eliminate that problem.

INTERNAL DOSIMETRY AND EXTERNAL MICROWAVE FIELD MEASUREMENTS USING MINIATURE ELECTRIC FIELD PROBES

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Bureau of Radiological Health, FDA

ABSTRACT

An optically-linked EM probe system was developed to meet the needs of researchers investigating UHF and microwave emissions from electronic products. In addition, it allows one to assess exposure levels accurately in biological-effects studies conducted in this frequency range. The probe's small size provides the maneuverability and high spatial resolution required to map fields with steep spatial gradients or those in confined areas, such as animal exposure restrainers. The fiber optically-linked telemetry system provides RFI immunity and enhances accuracy below 1 GHz, by eliminating cable scatter. The probe system was not developed as a replacement for commercially available isotropic "hazard survey meters" which are hand-held portable instruments. It was developed for more specialized laboratory measurements where present probes were not appropriate due to their physical size or hard-wired readout. A detailed example is presented in which the system is used at 450 MHz as à field mapping device to evaluate an animal exposure chamber. Fields within simulated and actual biological specimens have been measured with the above probe. A smaller version of the probe is discussed which can provide a practical means for measuring internal fields in living test animals exposed to external electromagnetic radiation.

ISOTROPIC PROBE

The basic elements of the probe are three orthogonal, miniature dipoles (2.5 mm long), each on its own substrate (Fig. 1). A beam-lead, zero-bias, Schottky diode shunts each dipole across its center gap, providing detection. The use of diode bonding wires is avoided since they would introduce a parasitic inductance, and an extra antenna element in the probe, creating a high-frequency resonance and also introducing antenna pattern degradation. A pair of high-resistance, thin film leads (97 kohm/cm) transmits the detected signal from the antenna to a second, lower-resistance pair of long leads. The detected signal is then fed to a preamplifier, suitably removed from the vicinity of the antenna elements. The high-resistance thin film leads, create a low-pass, distributed filter composed of a highly lossy, series inductance with distributed interlead capacitance. They are effectively transparent to the RF or microwave field under study. An "I-beam" configuration was devised for the miniature probe to allow three planar sub-strates to be placed very close together and yet provide minimal interaction between orthogonal dipoles (1). The center dipole's axis forms a line joining the centers of the other two dipoles (Fig. 2) so that all centers are colinear. Each substrate is 3 mm wide, 6.5 cm in length, and is 0.6 mm thick. A 30-cm epoxy-fiberglas "I-beam" frame supports the three substrates and provides each alumina substrate with an extended pair of medium-resistance

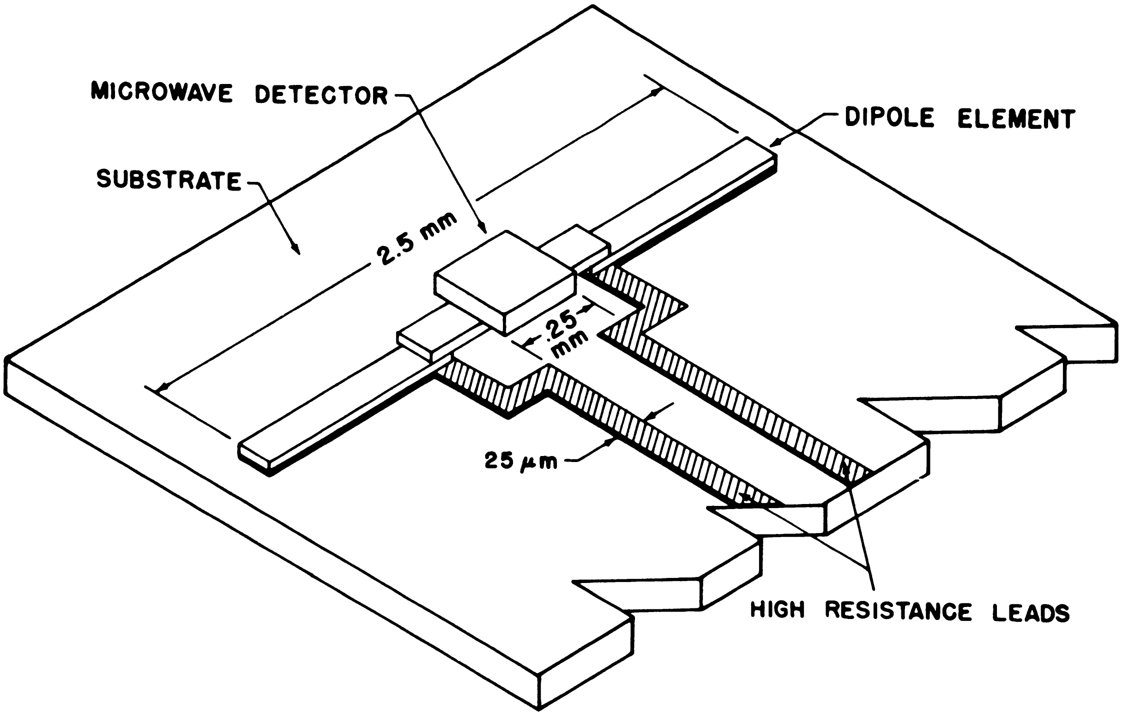

MICROWAVE DETECTOR
DIPOLE ELEMENT
SUBSTRATE
2.5 mm
.25 mm
25 μm
HIGH RESISTANCE LEADS

Figure 1. Dipole/detector substrate.

ORTHOGONAL DETECTOR ARRAY

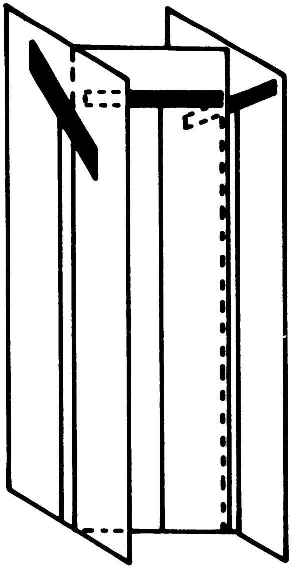


Figure 2. "I-beam" substrate configuration.

signal leads. This frame is fabricated from a commercially available material, Micaply Ohmega; (Mica Corporation, Culver City, California) and provides etched, "microwave transparent" leads, 500 ohm/cm, 0.5 mm wide, spaced 0.5 mm apart (Fig. 3). The bottom 24 cm portion of the epoxy-Fiberglas "I-beam" is housed in a plastic tube with a 1 cm outer diameter. A more recent version of the probe has been produced using a specially etched, 30 cm substrate containing a dipole, high resistance leads, and medium resistance leads, all on a single epoxy-fiberglas assembly. A comparative evaluation of these two designs will be presented later in this paper with emphasis on antenna patterns and the resulting degree of isotropic response.

Recent developments led to the creation of a zero-bias (low-barrier) beam-lead Schottky diode (Aertech Industries, Sunnyvale, California). Use of this device gave a dramatic increase in sensitivity (10 to 16 dB) with respect to previously used standard Schottky diodes and eliminated static charge noise, since the diode terminates the high resistance lead pair in a d.c. resistance of 5 to 10 kilohms.

OPTICALLY-LINKED TELEMETRY SYSTEM

A battery-powered, microminiature telemetry transmitter was developed to amplify the detected output voltages from each dipole and to convert the data to a digital form using voltage-to-frequency conversion techniques. An infrared light-emitting diode is then driven by the digital pulses, transmitting information on an optical fiber. Three such channels carry data over a single 4.6 meter optical cable bundle. For each channel, data is reconverted to an electrical replica of the detector voltage in a remote receiver. An analog receiver allows direct observation of DC to 2 KHz modulation of the field under study. Recently, an all-digital receiver was completed with a 3 digit L.E.D. display of the time-averaged field $|E^2|$. The system is illustrated in figure 4. When compared to a conventional, shielded cable, the optical link eliminates scatter from the metallic cable and minimizes RF interference in the system's electronics. Use of the system has shown that improved performance results as follows:

a. No long, metallic cable is present to alter the field under study or create scatter. This is especially important at frequencies below 1 GHz where cables may be close to the probe's detection elements, in terms of wavelength. When the electric field orientation is unknown, it is impractical to try to orient the readout cable for minimum interaction with the field.

b. RF interference, entering the preamplifier through its output cable, is eliminated. In conventional hard-wire links, the effectiveness of RFI filters and shielded cables is not always sufficient to completely eliminate pickup problems especially, when very small dipoles are used and high gain is required in the system's preamplifier. When a probe is used with a long metallic readout cable, RF pickup on the readout cable often causes erroneous probe readings. This was the case, below 1 GHz, for early versions of this system which used a hard-wired readout cable.

c. Optical fibers and light-emitting diodes allow a very small preamplifier-to-cable interface to be made in an RFI-free manner, one which is well suited to microminiaturization. No crosstalk between channels occurs within the multicable, fiber-optics bundle emerging from the telemeter. The highly flexible fiber-optic cable bundle (8 mm in diameter) allows complete maneuverability in tight quarters or in conjunction with antenna rotators, etc.

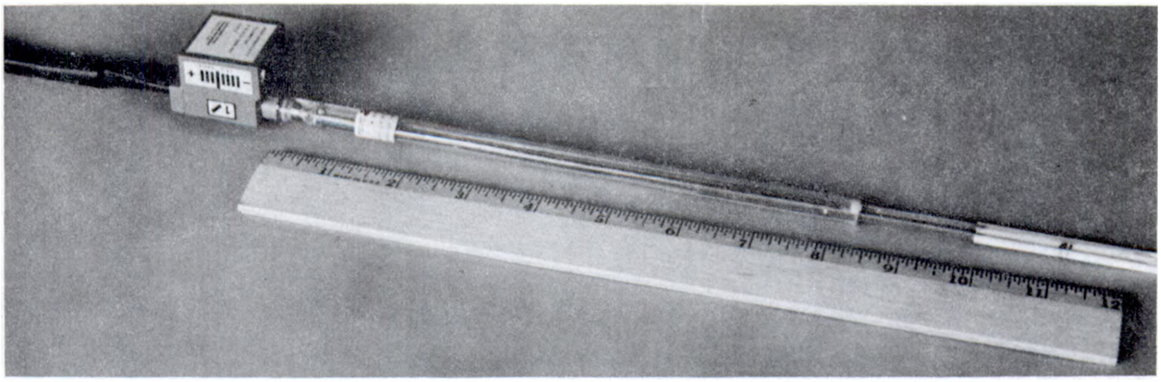


Figure 3. Three axis probe (with 12 inch ruler).

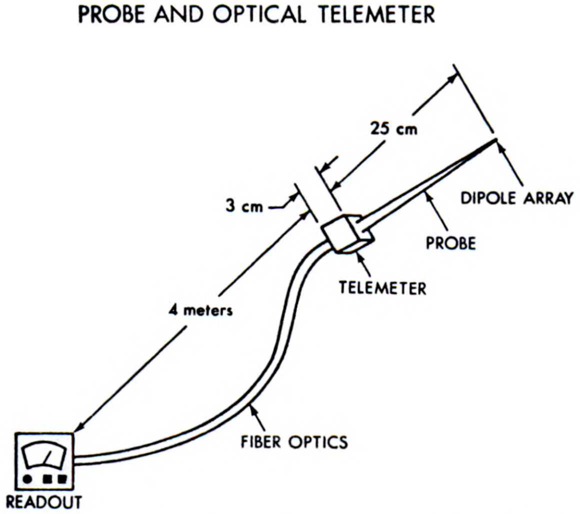

PROBE AND OPTICAL TELEMETER
25 cm
3 cm
DIPOLE ARRAY
PROBE
TELEMETER
4 meters
FIBER OPTICS
READOUT

Figure 4a. Probe/telemetry system configuration.

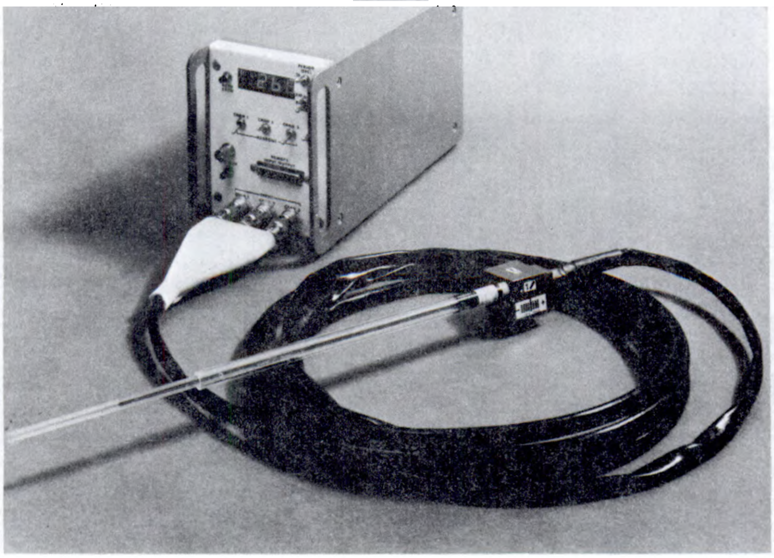


Figure 4b. Probe with digital telemetry receiver.

In a series of experiments, in an anechoic chamber scatter from a 1 meter cable aligned with the electric field, was found to introduce a probe error of ±0.75 dB at 915 MHz and ±1.5 dB at 450 MHz (2). In contrast, the scattering error from the metallic telemetry transmitter housing was less than ±0.2 dB over the same frequency range.

ISOTROPIC PROBE SYSTEM EVALUATION

The three-dimensional E-field probe with optical telemetry system was evaluated in an anechoic chamber under plane-wave irradiation from 200 MHz to 12 GHz to determine the degree of isotropic response. Absolute calibrations were also performed. From 100 to 500 MHz, calibration was performed in a special TEM transmission-line calibration cell. At higher frequencies, far-field calibrations were performed in anechoic chamber. Frequency response is shown in figure 5. The steep drop in probe output versus frequency that occurs below 1 GHz is due to the relatively high impedance (capacitive reactance) of the electrically small antenna, coupled with the low diode impedance (due to diode leakage current). No resonant phenomena should occur below 20 GHz, since the dipole length and diode capacitance are both very small. The effective height of the dipole at 50 GHz is approximately one half wavelength. No tests were performed above 12 GHz due to equipment limitations, and the lack of a specific application by the developers.

The sensitivity of the system allows the detection of a 20 µW/cm2 (8.7 V/m) field at 2.5 GHz with a 10 dB signal-to-noise ratio in a 1-Hz bandwidth or a 200 µW/cm2 (27.5 V/m) field in a 2 KHz bandwidth. Dynamic response (linearity) of the probe is illustrated in figure 6. This sensitivity is comparable to presently available "microwave hazard" probes whose size (and spatial resolution) are considerably larger than the minature probe. Because the dipole impedance increases with wavelength, low frequency sensitivity is reduced when the low-impedance zero bias diode is used as a detector. The good UHF and microwave performance afforded by the zero bias detector is more desirable than an increase in lower frequency response which might result if other detectors were utilized. Square law response (E^2) of the diodes does not exist for signals greater than 10 to 40 mW/cm^2, depending upon individual diode sensitivity and the frequency of irradiation. Above this level, the response of the diode approaches a linear proportionality with respect to E, the electric field strength. Therefore this probe is not capable of measuring short radar pulses with high peak power. The diodes are highly resistant to burnout at levels below several watts/cm2 (several kilovolts/meter). The response to amplitude modulated fields is such that 2KHz sinusoidal modulation is readily followed. Temperature response was obtained in a temperature chamber which was lined with microwave absorber. A slot antenna irradiated the probe in the chamber while relative response was obtained over the 0° to 55° C range. The change in probe sensitivity was found to be approximately 0.5 percent per degree Celsius.

Recently, several new single-axis microwave probe prototype substrates were designed and evaluated to provide a more isotropic response from the three dimensional antenna array. The basic improvements with respect to previous BRH probes were in the high resistance lead designs as shown in figure 7. These modifications were incorporated in an effort to reduce the antenna pattern degradation occurring in the previous probes. This degradation produced an isotropicity error of up to ± 3 dB at the lower frequency portion of the previous probe's operating range, when the sum of the orthogonal dipoles outputs were taken while the probe was rotated in a plane wave field. The new probe designs included one dipole on an alumina substrate ($\epsilon_r = 9$) with 250 ohm/square resistive lines and $\Theta = 45°$, where Θ = the angle of the dipole elements with respect to the probe handle, and one dipole on a "micaply ohmega" epoxy fiber-

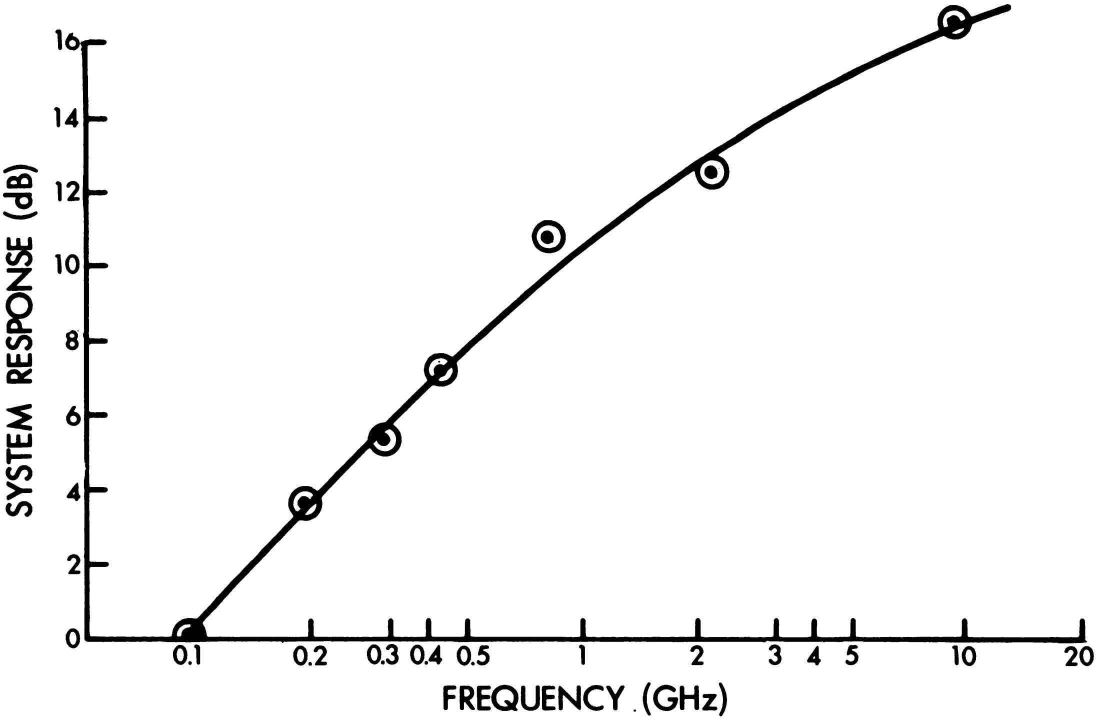


Figure 5. System frequency response.

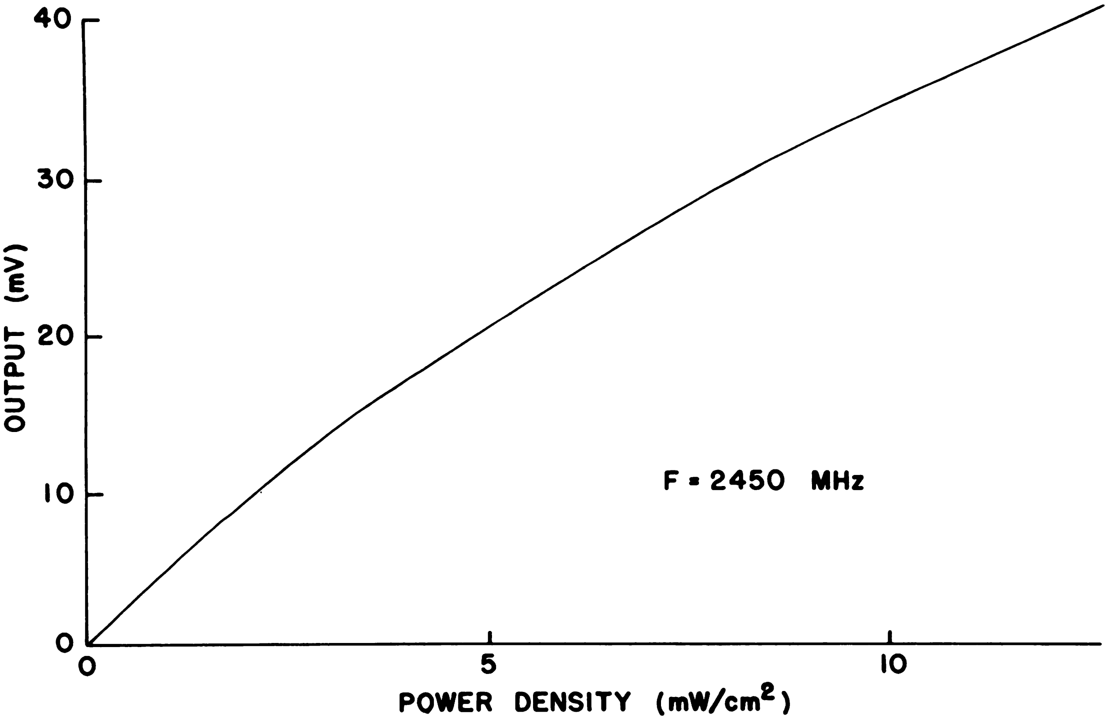


Figure 6. System dynamic response.

glas substrate ($\epsilon_r = 4$) with thin film resistive material (100 ohm/square) and Θ = 45°. All dipoles were 2.5 mm total length. Earlier probes had an alumina substrate with 100 ohm/square resistive lines and Θ = 45°.

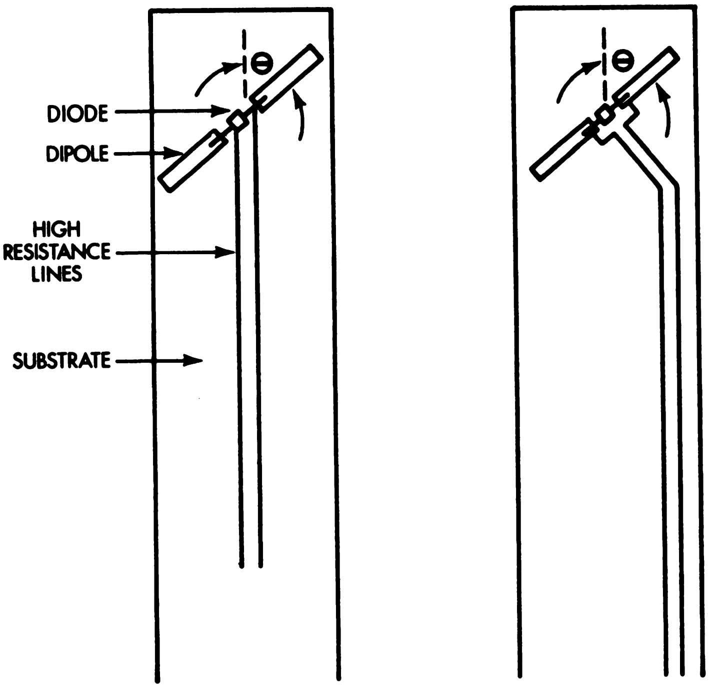
Evaluations of antenna patterns were performed over a frequency range of 200 MHz to 10 GHz. The pattern evaluation involved the rotation of the probe in a linearly polarized electric field as shown in figure 8. To simulate a three-axis probe, the single axis probe design, which would be used as a "side dipole" (two required per 3D probe) was evaluated in two orientations. Both orientations were identical, with the probe handle positioned the same way for each pattern, but with the probe rotated 180° about the handle axis (Fig.9). Since the center axis of the BRH probe has always had a nearly "perfect" dipole pattern, its pattern was only taken once with an older 3D probe. This data was utilized to aid in the prediction of the overall performance of a hypothetical "improved" 3D probe, incorporating each of the new substrate designs.

Pattern data was taken first on an "old" 3D probe at 2450 MHz. Results shown in figure 10 illustrate the two side-dipole patterns. No rotation of the probe body about its handle axis was required to obtain the two side-dipole patterns. An epoxy fiberglass version of this previous design had an identical pattern when compared to the original alumina probe pattern. The new epoxy-fiberglass probe was evaluated and, as shown in figure 11, the results represent a substantial improvement over the previous design. If one sums the two patterns manually, the variation (expressed as the minimum-to-maximum ratio) in a single plane, is less than 0.75:1 at 200 MHz, 0.90:1 at 2450 MHz, and 0.95:1 at 10 GHz. In contrast, the old design has a ratio of 0.45:1 at 2450 MHz, and an even greater variation at lower frequencies. The new alumina probe design does not possess a significantly improved pattern with respect to the previous design. This implies that the dielectric constant of alumina ($\epsilon r = 9$) degrades the antenna pattern when compared to the epoxy fiberglass ($\epsilon_r = 4$) pattern. In addition, lead geometry is important to reduce coupling to the dipoles. This assumption is verified by comparing the old and new epoxy-fiberglass probes where only the lead orientation was changed, resulting in significant pattern changes.

Preliminary pattern experiments using the above probes, implanted in muscle-equivalent material, have yielded very interesting results. The antenna pattern is affected significantly by implantation in this high-dielectric-constant ($\epsilon r = 50$) material. An alumina probe for implantation was studied in air and in muscle equivalent material with radiation applied from above the rotator (propagation vector S_2 in figure 8). The pattern in muscle material was found to be nearly ideal. However, when not implanted, the pattern nulls appeared shifted by 15°. This leads to a hypothesis that microwave "refraction" occurs in the substrate material whose dielectric is higher than the surrounding media. Therefore, a very low dielectric constant substrate is desirable for free space probe design.

SUMMARY SYSTEM PERFORMANCE SPECIFICATIONS

- Frequency Range 0.2 to 12 GHz
  Frequency Response: approximately 2 dB/octave (see Fig. 5)
- Measurement Range: 20 µW/cm2 to 20 mW/cm2 equivalent
  plane wave power density. (8.7 to 275 Volts/meter.)
- Isotropic Response (using "improved" epoxy-fiberglass substrate design)
  1 to 12 GHz: ± 1 dB
  0.2 to 1 GHz: ± 2 dB or less
- Absolute accuracy: ± 1 dB at calibration points


DIODE
DIPOLE
HIGH RESISTANCE LINES
SUBSTRATE
Θ
Θ

(a) PREVIOUS DESIGN

(b) NEW DESIGN

Figure 7. Probe design modifications.

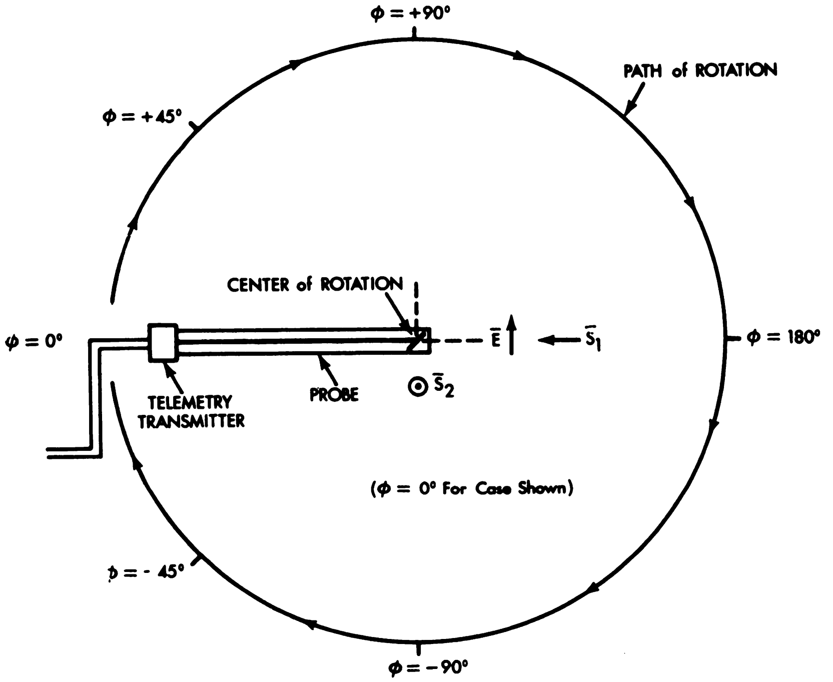

$\phi = +90°$
PATH of ROTATION
$\phi = +45°$
CENTER of ROTATION
$\phi = 0°$
$\bar{E}$
$\bar{S}_1$
$\phi = 180°$
TELEMETRY TRANSMITTER
PROBE
$\bar{S}_2$
($\phi = 0°$ For Case Shown)
$\phi = -45°$
$\phi = -90°$

Figure 8. Probe pattern measurement technique.

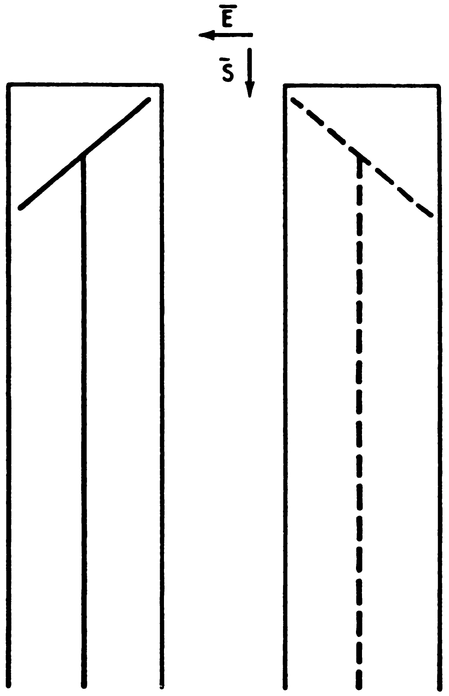


Figure 9. Rotation of probe about handle axis.

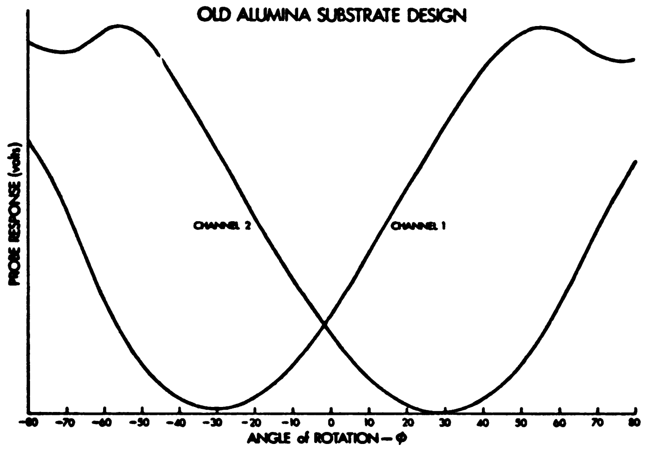


Figure 10. Probe response pattern.

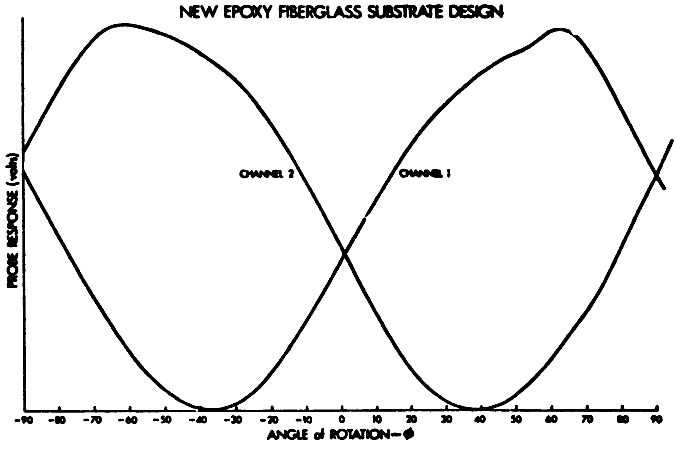


Figure 11. Improved probe response pattern.

- Temperature Response: 0.5 percent/°C over the 0°C to 55°C range.
- Detection Bandwidth: 2KHz (200 μsec rise time)
- Spatial Resolution of probe sensor - approximately 3 mm.

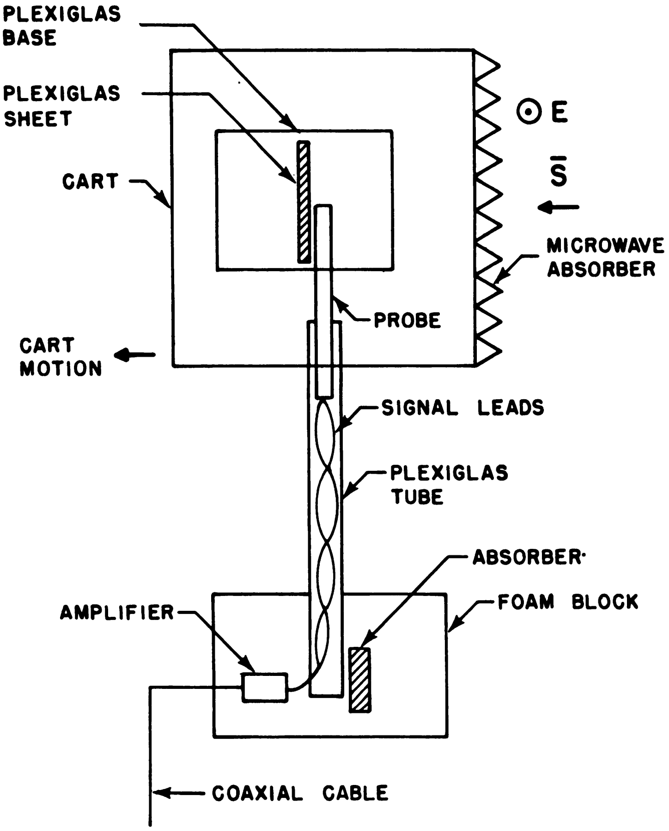
MEASUREMENT OF EXTERNAL AND INTERNAL RF AND MICROWAVE FIELDS

The three-dimensional E-field probe has been used to study scattering and field distributions near a Plexiglas sheet with application to biological effects experiments in which animal holders made of Plexiglas were used (3). At 2450 MHz, 1/8" plexiglas sheets have been found to create field perturbations of up to 3 dB when aligned either normally or parallel to the incident propagation vector in a plane wave field (Fig. 12). Near fields, with steep spatial gradients, a few centimeters from microwave diathermy applicators have been mapped (4). In many of these applications the small size and maneuverability of the probe, plus its non-metallic optical data link, proved invaluable, allowing simple setups and positioners to be utilized while maintaining good system measurement accuracy.

E-field distributions within simulated biological tissue, irradiated by microwave energy, can be directly measured. A correction factor, with respect to free-space calibration, is required when implanting the three-dimensional probe (5). This varies from less than 2 dB at 2450 MHz, to more than 4 dB at 450 MHz. This is due to low diode impedance relative to antenna impendance. As an example of this type of measurement, fields existing in a 3.3 cm radius sphere of muscle-equivalent material are shown. This data was taken with an epoxy-encapsulated probe, mechanically scanned from the rear to the front of the sphere, passing through its center; where a focal point exists. Good agreement exists between the theoretically-predicted field distribution and the measured data (Fig. 13). The sensitivity of the present probe is sufficient to measure internal fields in finite biological specimens irradiated under "non-thermal," plane-wave exposure conditions (a few mw/cm2).

An example of a practical internal and external field measurement project will be discussed in detail. The project involved the evaluation of a 450 MHz exposure chamber used by the Brain Research Institute of UCLA for low-level bioeffects studies. The chamber consists of a 1.5 meter high x 1.5 meter wide, by 80 cm deep exposure area, lined with microwave pyramidal absorbers, fed by a metal screen-lined pyramidal "horn" whose aperture forms the front surface of the exposure zone. A series of measurements were performed in the chamber, with and without experimental animals in the exposure zone. These measurements were designed to evaluate several aspects of the fields including: (a) uniformity of the electric field within the unoccupied exposure zone including standing waves and other spatial gradients, (b) polarization of the electric field vector within the exposure zone, and (c) the change in field parameters in the exposure zone created by the introduction of test animals and restraining devices. The ideal situation would be a "plane wave" exposure situation, that is, a linearly polarized electric field, with minimal variations in magnitude along the axis of propagation and in any plane normal to this axis.

The small size of the BRH probe provided good spatial resolution of the fields during the series of measurements, and allowed the mapping of areas within the animal restrainers and the chamber to be performed easily, even in small, confined areas. The fiber-optics bundle was flexible enough to allow complete maneuverability in these restricted quarters. The advantages of the non-metallic, optical fiber readout cable over conventional wires are that minimal reflections are caused by the telemetry transmitter and fiber optics,


PLEXIGLAS BASE
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CART
⊙ E
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MICROWAVE ABSORBER
PROBE
CART MOTION
SIGNAL LEADS
PLEXIGLAS TUBE
ABSORBER
AMPLIFIER
FOAM BLOCK
COAXIAL CABLE

Figure 12a. Plexiglas scatter experiment (normal orientation).

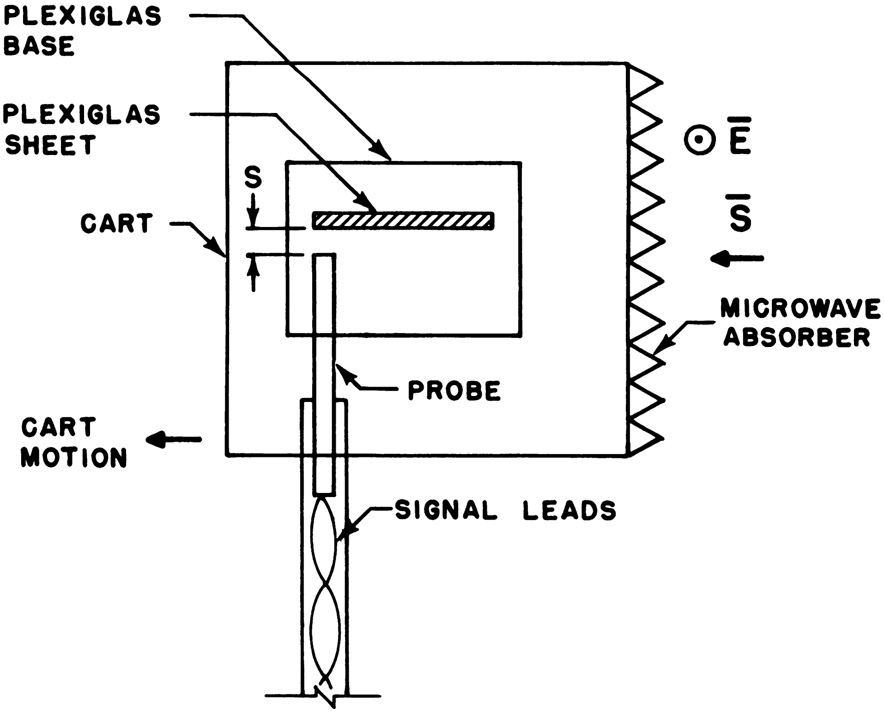
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Figure 12b. Plexiglas scatter experiment (parallel orientation).

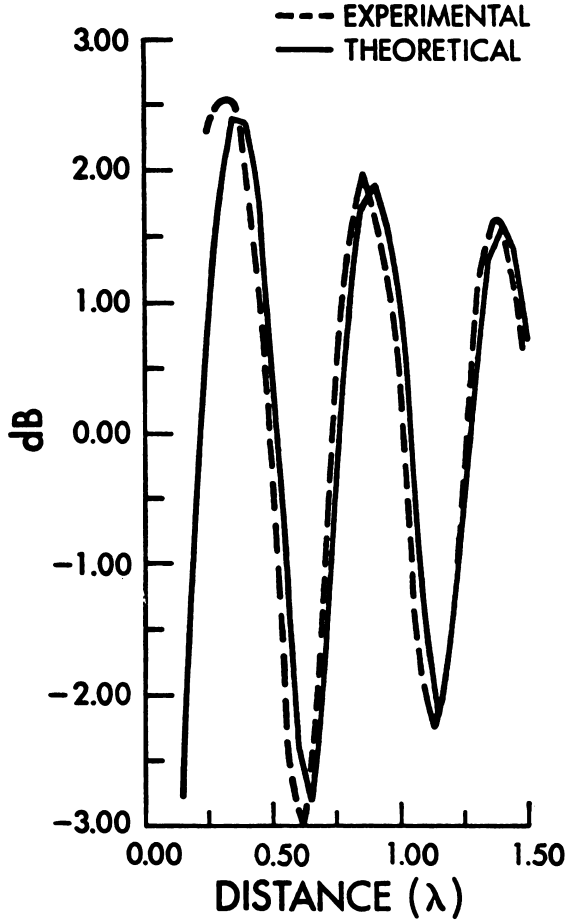


Figure 12c. Scatter data (normal orientation).

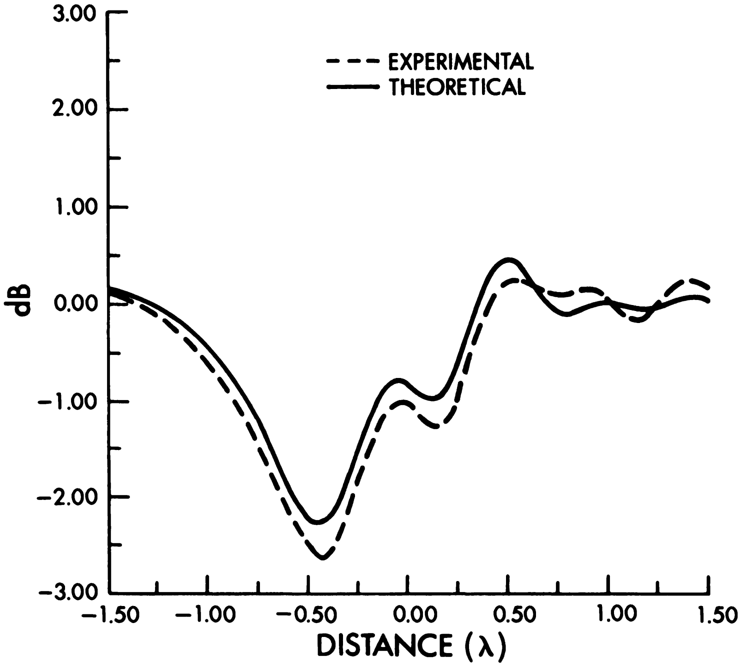


Figure 12d. Scatter data (parallel orientation).

compared to those created by a long metallic cable. Previous to this measurement approach, a commercial hazard meter was used with a metal cable and readout box concealed in a large block of microwave absorbing material. This unit proved cumbersome, and point measurements were very time consuming when taken in the chamber's exposure zone. The use of the BRH miniature probe and its optical telemetry system contributed directly to the overall accuracy of the measurements, not only by reducing errors, due to scatter, but by allowing a much greater amount of data to be taken in a reasonable amount of time.

Measurements were performed first in the empty chamber using a reference grid system marked off with masking tape on the floor of the exposure zone. Point measurements were taken at grid line intersections, at various heights for both "vertical" and "horizontal" electric field orientations which were established by the position of the coaxial-to-waveguide feed for the chamber. Additional data was taken for both the empty and occupied chamber by performing continuous scans with the probe, which was mounted on a long wooden pole. The pole length (approximately 2 meters) was sufficient to allow the chamber to be scanned while the experimenter was outside the chamber, holding the pole which was passed through an open door. It was experimentally demonstrated that the open door did not alter the fields in the exposure zone, and that no hazardous fields existed in the area occupied by the experimenter. Continuous scan data was recorded in real time as a function of distance, which allowed standing wave minimas and maximas to be quantified, as well as the field variations which existed in the various planes normal to the axis of propagation.

The unoccupied chamber was found to possess a relatively uniform, linearly polarized electric field, in a 50 cm x 150 cm plane, normal to the axis of propagation. Variations of the field (E^2) did not exceed approximately ±10 percent in any one plane perpendicular to the axis of propagation, from the rear wall of the chamber, to a position 80 cm ahead of the rear wall. Standing waves along the axis of propagation created variations in the value of E^2 of approximately ±20 percent along the center axis, over the 80 cm distance. The larger uniform dimension of the "quiet zone" plane was aligned with the electric field vector and was dependent on the waveguide feed orientation, not the chamber itself. When occupied by a monkey, (75 cm head-to-toe, when sitting in a plastic chair), the reflections caused variations in E^2 of ±40 percent (maximum at the pelvis). Fields directly behind the monkey dropped to a few percent of the incident field (both vertical and horizontal) implying a significant absorption of energy. No estimate of internal energy deposition or fields in the head or extremities was made. Internal electric field measurements could be made utilizing a small, insulated E-field probe, as they were for the case of a cat in this same chamber, as follows.

A plastic and wooden apparatus was designed and constructed by Wakefield, Bawin and Adey of the Brain Research Institute (UCLA). The head of an anesthetized adult cat was thus supported in a non-perturbing manner, facing the incident propagation vector of the horizontally polarized field. Measurements were taken with an insulated, single-axis, alumina substrate, 2.5 mm dipole probe at three sites between the cerebral hemispheres. As expected, by rotating the probe, it was determined that the polarization of the internal electric field was identical to that of the external field. This is similar to the findings obtained in the theoretical analyses of induced fields in multilayered dielectric spheres, where the polarization of the fields remained constant throughout the spheres. Figure 14 gives internal field strengths relative to external fields. Post-mortem measurements in the animal's brain at the same sites as were previously used, indicated that virtually no change in the internal field strengths occurred immediately after death.

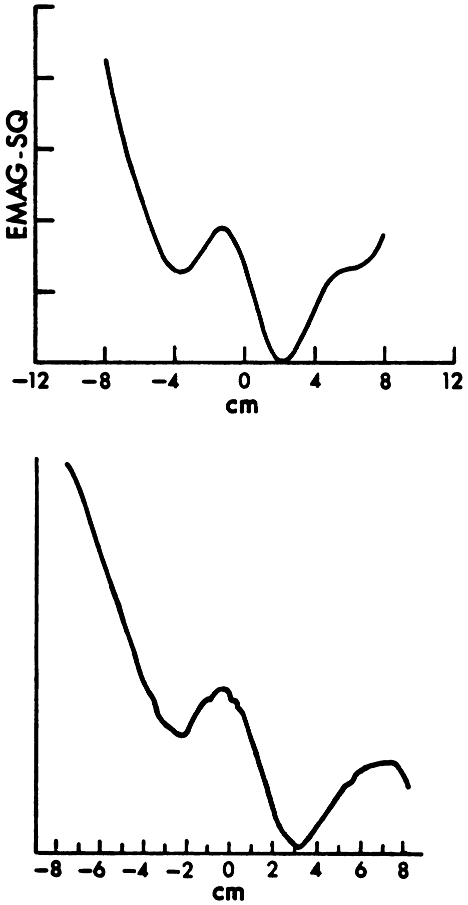
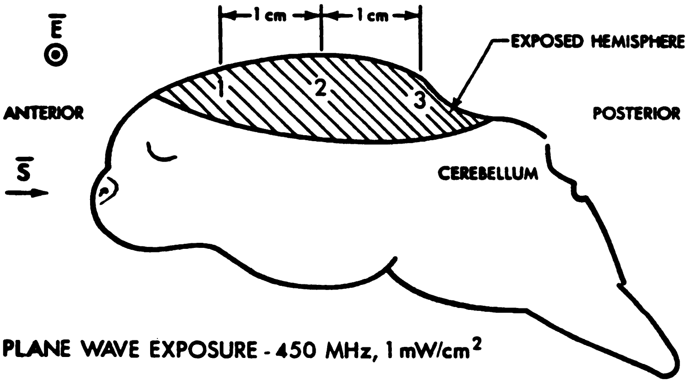


Figure 13. Theoretically predicted (top) and measured (bottom) 450 MHz fields in brain-equivalent sphere (scan line parallel to external propagation vector).

IMPLANTATION SITES


1 cm
1 cm
$\bar{E}$
ANTERIOR
$\bar{S}$
1
2
3
EXPOSED HEMISPHERE
POSTERIOR
CEREBELLUM

PLANE WAVE EXPOSURE - 450 MHz, 1 mW/cm^2

IMPLANTATION SITE	INTERNAL FIELD STRENGTH
No. 1 (Depth 0.5 cm)	16 V/m
No. 2 (Depth 1.5 cm)	14 V/m
No. 3 (Depth 1.5 cm)	6 V/m

Figure 14. Measured data obtained with implantable probe in living cat's brain (± 2dB uncertainty).

The epoxy-encapsulated probe utilized had a cylindrical cross section of approximately 4-5 mm. This size made further implantation studies in the brain impracticable because of the possibility of extensive tissue damage and associated bleeding. A preliminary design has been developed for a 1.5 mm dipole probe, deposited on a flexible plastic substrate and possessing a cross-section, when encapsulated, of approximately 2 mm. This design should allow more flexibility in both the selection of implantation sites and the elimination of the complete immobilization of an animal during irradiation.

ACKNOWLEDGEMENT

The original fabrication of miniature electric field probes was performed by Dr. J. Abita and his staff at Johns Hopkins University, Applied Physics Laboratory. The analysis of the effects of implantation of the probe in biological media was performed by Dr. A. Cheung of the University of Maryland.

The author wishes to acknowledge the substantial contributions of Mr. M. Swicord, Chief, Electromagnetics Branch and staff members W. Herman and D. Witters all of the Division of Electronic Products, Bureau of Radiological Health.

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OPEN DISCUSSION

DR. GLASER: I am glad that implantation is where we are now going to be able to get E fields. I am wondering, referring to the last slide that you showed which indicates both E fields, whether that could be coupled with temperature measurement and whether you might be thinking about a probe that will give you E field and temperature.

MR. BASSEN: I think the two techniques are complementary in the sense that an animal has an active thermregulatory system and it would be difficult, although possible, to get instantaneous measurements of the E field or absorbed dose distribution in a living animal; and I would not want to combine the two into a single instrument because you would compromise the design of both. I think that one point to be made is that a tool that can provide measurements within a stated degree of accuracy is better than no tool at all; that is, for in situ measurements where actual biologists can just take this device, measure fields in the various specimens in his own laboratory, and get readings which have a finite degree of error, but a known degree of reliability about them.

DR. CARPENTER: I might follow the request of Zorach Glaser: can't you develop an instrument that will measure the power density, measure the temperature, and write the paper? I would like to comment that the use of this instrument saved us from making a serious error. We had been measuring an incident field with a NARDA 8323 probe which is 3 dipoles. It is necessary to measure this behind a dielectric lens if you are going to use enough power to produce difficulties in the eye; a far field exposure of that type kills a rabbit. So when you use a dielectric lens, you know the dielectric lens concentrates the RF at a certain point behind the lens. You measure it behind the lens with an 8323 NARDA, and you can move it around within fairly close limits and it will always say the same thing. It measures an area two inches in diameter and when that is applied to a rabbit eye, which is 18 millimeters in diameter, you average the two inch field, and say that is what the eye is getting, you are all off. So we used this beautiful probe (I call it the Bassen probe) to measure that field covered by the NARDA probe two inches square, and we measured it every five millimeters which told us a great deal. We found that at 10 gigahertz that the concentration was very good and that it was a concentrated field that fell off rapidly. However, at 2450, the same lens was not concentrating; and if you averaged a two inch area, you came fairly close to what you would get if you averaged the two centimeter area. But in comparing that with a 10 gigahertz, would have been seriously off if we had relied on the 8323. I think this probe is a beautiful thing to check other probes with; it gives you a feeling of security.

INFRARED EMITTANCE OF ELECTROMAGNETIC PHANTOM MATERIALS AND INFRARED TRANSMITTANCE OF POLYETHYLENE

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Department of Radiology
Tucson, Arizona
and
G. Kantor
Division of Electronic Products
Bureau of Radiological Health, FDA

ABSTRACT

Methods of obtaining quantitative thermometric data with a thermographic camera are discussed. These include the assessment of errors associated with the camera itself and of difficulties arising from the nature of the object to be measured. The latter refer to determinations of the "penetration of depth" of infrared radiation emitted by the object (how "thick" is the surface?); measurement of the transmittance of "windows" in front of the objects; and measurement of the object's emittance under somewhat adverse circumstances. Finally, an example of a temperature measurement is given. Practical considerations of thermometry are emphasized as opposed to radiometric measurements (such as spectral properties). Examples are drawn from measurements involving tissue-equivalent electromagnetic phantom materials.

INTRODUCTION

Thermographic cameras are used widely for observing thermal distributions. Commonly the purpose is to determine anomolous thermal patterns which may be indicative of troubles such as the presence of tumors in breasts (1) or of failing components in an electrical circuit. Nevertheless, many situations exist for which the goal is to obtain quantitative temperature measurements with a resolution close to the minimum detectable temperature difference of the thermographic camera. Measurements of the thermal pattern induced in tissues by some heating method would be an example of this. For quantitative temperature measurements, a knowledge of the characteristics of the camera itself is necessary. These include assessment of the temperature equivalent noise level, a determination of systematic errors and a calibration of the specific thermographic camera. Typical calibration curves supplied with the cameras are not really adequate for precision thermometry. The infrared radiometric properties of the object to be measured must be known also. The penetration depth in the object from which infrared radiation will escape and be detected by the camera should be determined. The transmittance of any window materials that are placed in front of the object must be known. Finally the emittance of the object itself must be measured. Following recent trends in radiometry (2,3,4) the "-ance" ending will be used to refer to a specific object (for example, emittance and reflectance). The ending "-ivity" will refer to intrinsic properties of an optically smooth surface of the material (for example, emissivity or reflectivity). The situation is quite analogous to that of resistance versus resistivity. Throughout the paper, practical considerations of thermometry will be emphasized rather than radiometric measurements. Examples will be drawn from measurements involving tissue-equivalent electromagnetic phantom materials.

The derivation of the equations governing the use of thermographic cameras is given in a more complete paper to be published elsewhere (5) as will the techniques used to calibrate the camera. However, it is useful to list here some of the equations in order to more fully understand the measurements. The total camera response i_1 to a specific subject s_1 at temperature T_1 is the sum of the radiation emitted, reflected, and transmitted by the subject:

$$i_1 = \varepsilon_1 \, I(T_1) + \rho_1 \, I(T_a) + \tau_1 \, I(T_b) \qquad (1)$$

Here ε_1, ρ_1, and τ_1 are the emittance, reflectance and transmittance characteristics of the subject and T_1, T_a and T_b are the subject, ambient and background temperatures. The I (T) represents the response of the thermographic camera to radiation which it receives from an ideal blackbody source. The laws of radiometry (2,3,4) will apply to this function. The characteristics of the source are treated explicitly in this formula. A second object will have a similar response, i_2. The camera is frequently and most accurately used to measure temperature differences between two sources or to measure temperature gradients on a single object; thus, the difference Δi_{12} can be represented as:

$$\Delta i_{12} = i_1 - i_2 = \varepsilon_1 \left(I(T_1) - I(T_2) \right) + (\varepsilon_1 - \varepsilon_2) \left(I(T_2) - I(T_a) \right) \qquad (2)$$

Here we have assumed that the object is sufficiently thick that no radiation from behind the object is transmitted ($\tau_1 = \tau_2 = 0$). We also have set $\rho = 1 - \varepsilon$. This is a familiar expression which involves both conservation of energy for radiation and the Helmholtz relation which states that the absorptance of incident radiation by an object equals the emittance of radiation by that object (under certain restrictions). Some features of this expression must be noted. Only temperature differences are given, such as $I(T_1) - I(T_2)$, so an absolute calibration of the camera is not necessary. Ambient temperature must be recorded in order to account for the radiation that is reflected from the surface. According to the derivation of equation (2), only diffuse background radiation is permitted. No provision is made for specular reflection of radiation emitted from a localized hot source in the vicinity.

If the camera is calibrated, that is, if the function I(T), or more particularly its derivative $\Delta I / \Delta T$, is known, we can determine $T_1 - T_2$.

CALIBRATION OF THE THERMOGRAPHIC CAMERA

The calibration of the camera is described in detail elsewhere (5), but some of the results are presented here in order to clarify the discussion of later measurements. Figure 1 shows the thermographic display of a commercial black reference source at 28° C in an ambient of 25.5° C. Figure 1A is a gray scale representation with white as hot. For this particular thermogram, a range of temperatures of approximately 10° C corresponds to the gray scale along the bottom of full black to full white. Figure 1B shows a temperature profile along the line indicated by the white fiducial marker in 1A. Temperatures are represented by vertical displacements according to the position along the white line. The central hot region is indicated as the flat plateau and the background temperatures are represented by the lower line on the two sides of the thermogram. In 1C, the temperature profile was taken through the gray scale at the bottom of 1A. This serves to calibrate the temperature profile line display in terms of the temperature units of the thermographic camera. For example, a 10° temperature difference in thermographic camera units corresponds to 6.25 divisions in the temperature profile display.

The limiting accuracy of the measurements, is determined by the minimum detectable temperature (or, the temperature equivalent noise level) plus the

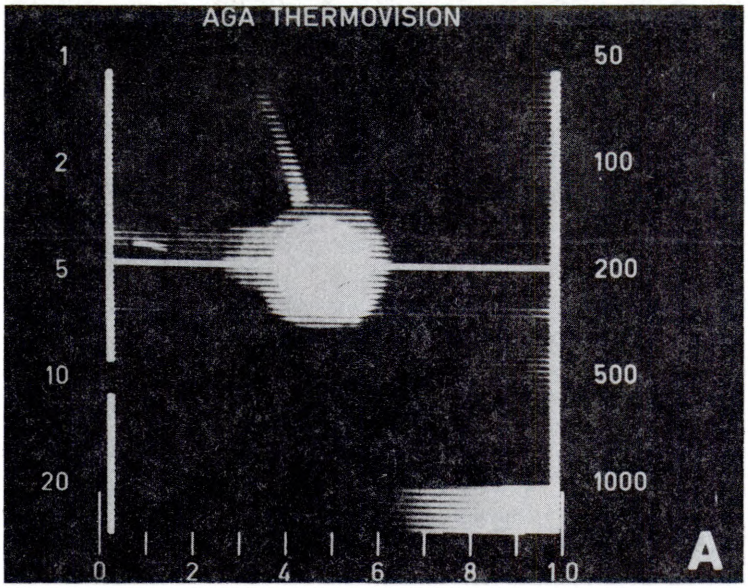


Figure 1. AGA thermogram of 28° C black reference source. Ambient temperature is 24.5° C.

Figure 1A. Two dimensional gray scale scan. The break in the white vertical line indicates the approximate full scale temperature range for gray scale shown at the bottom.

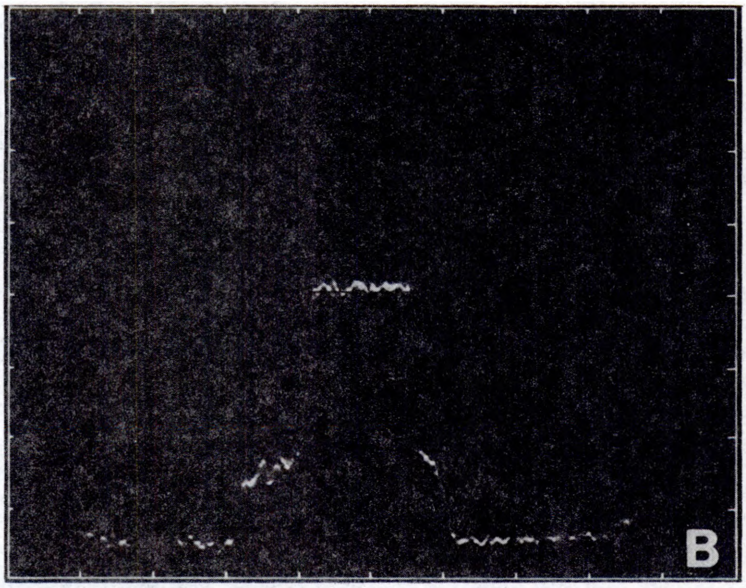


Figure 1B. Temperature profile along line indicated by horizontal white fiducial marker in A. Vertical displacement is proportional to temperature.

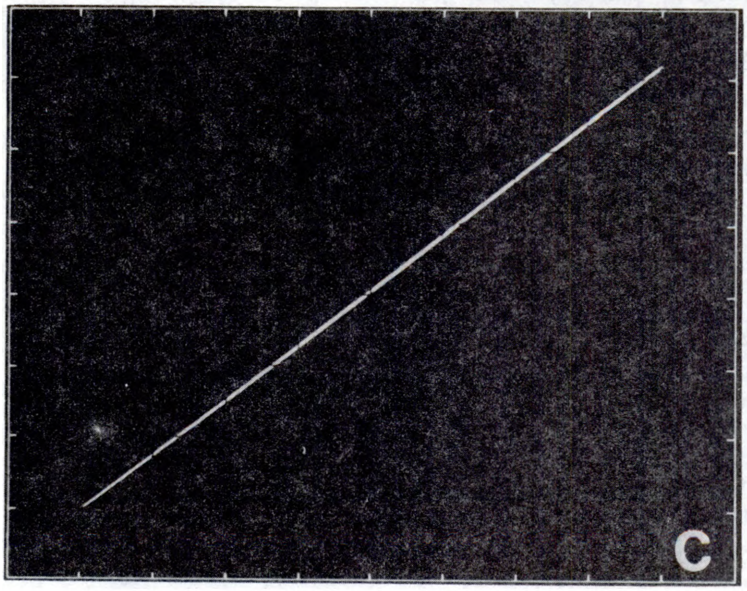


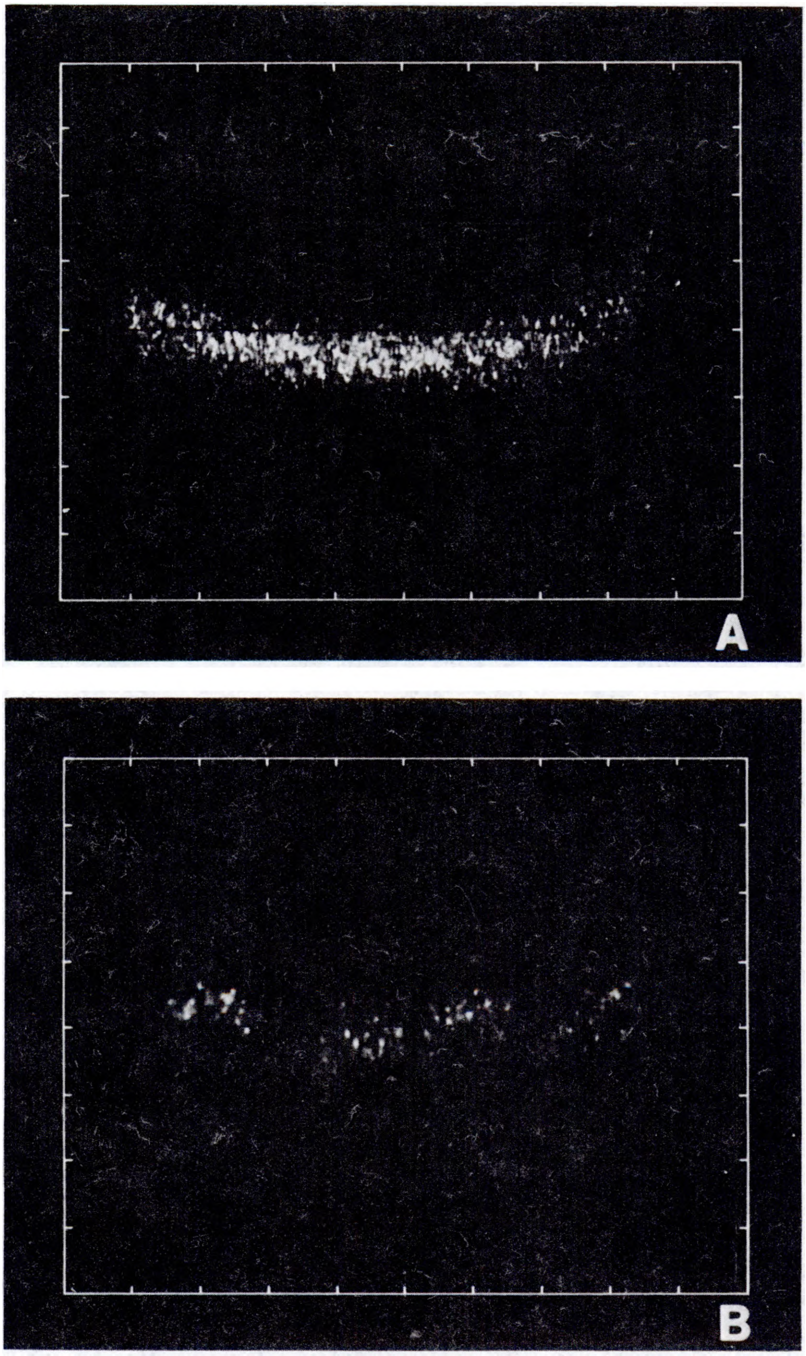
Figure 1C. Temperature profile along gray scale at bottom of thermogram A. Serves to calibrate temperature profile display in terms of camera parameters (graticule divisions/camera "temperature" units); in this case, 6.25 divisions/full scale setting.

magnitude of any systematic errors that may be present. Figure 2A shows a thermal line profile taken with a cover at a uniform temperature placed over the aperture of the camera. A straight, horizontal line would have been expected but, in fact, some aberration exists at each edge. The overall sensitivity of the camera for this photo is 2° full scale and the peak-to-peak noise level is 0.3° C. At the edge of the field, beyond ± 3 divisions from the center, the profile curves upwards and results in a temperature error of +.16° C. Figure 2B is another way of demonstrating the same error. A multiple exposure was taken of a black reference source (Fig. 1A) such that it appeared at various positions in the field of view. As can be seen, the thermograms taken with the reference source on the edge of the field of view appear to be warmer by approximately 0.16° than those taken in the center.

The calibration of the camera consists of interpreting the camera display, ΔI, as a function of the temperature difference ΔT. The procedure is to view two black reference sources at temperatures T_1 and T_2 and measure the corresponding difference $\Delta i_{12} = I(T_1) - I(T_2)$. (Set $\epsilon_1 = \epsilon_2 = 1.0$ in Equation 2). In order to eliminate errors associated with the source, it is important that blackbody sources (ϵ=1.0) be used. One blackbody was constructed according to the design suggested by Bedford (6). The true radiant temperatures of a commercial source then were measured by comparison to the blackbody using the thermographic camera. Thus, two black sources were established. The result of the calibration of the camera against the two sources is shown in figure 3. The Y-axis gives $\Delta I \;/\; \Delta T$ which is the ratio of the camera response to the temperature difference between two the sources. The X-axis is plotted in terms of the mean temperature, $(T_1 + T_2) / 2$. The various symbols correspond to different temperature intervals ranging from 2° C up to 8° C. When plotted in this fashion, all data points lie along the same line. This graph demonstrates that at lower temperatures, near 25° C, the camera response is only 80 percent that of the true temperature difference. A 5° C temperature rise, from, say 23°-28° C will appear as only 4°. Conversely, near body temperature, 37° C, an apparent 5° temperature difference between 34° C and 39° C, in fact, would be equivalent to a 4° C difference. This can be important in certain measurements, especially when 0.5° C to 1° C is critical, as in hyperthermia or in quantitative calculations of power absorption.

A few other sources of errors must be recognized in measurements with thermographic cameras. The first is to verify that the source is large compared to the spatial resolution of the camera. One procedure for testing this is to blacken the knife edges of vernier calipers and place them in front of a hot source. The slit width, for which the apparent temperature difference between ambient and the hot source is one-half the true difference, is a measure of the spatial resolution at object distance from the camera. This width divided by the distance to the camera, gives the spatial resolution in terms of milliradians, typically 1.3 milliradians for the systems we have used. This question has been considered in more detail by Macey and Oliver (7).

Another error can arise from viewing the source at an oblique angle. Watmough, Fowler and Oliver (8) have shown that for diffusely emitting materials, the error is not significant (0.1° C) for angles less than 30° but becomes important (several degrees Celsius) for angles greater than 50°. Finally, care must be taken to insure that the camera optics are clean and that no localized hot sources exist in the vicinity of the measurement. Incandescent lights, instrumentation racks or even the observer himself can reflect specularly from the surface of the object and be detected by the camera.



Figures 2A and 2B. Thermograms demonstrating the errors in the temperature reading for measurements made near the edge of the field of view. The thermographic system is set approximately for 2° C full-scale sensitivity and indicates a peak-to-peak noise level of 0.3° C. Figure 2A is taken with an isothermal cap over viewing aperature. Figure 2B is a multiple exposure taken such that the same reference source appeared in different positions of the field-of-view.

MEASUREMENT OF MATERIAL PROPERTIES

Knowledge of the infrared properties of materials such as emittance, reflectance, penetration depth and transmittance is important for determinations of the temperature of the object. Reflectance and emittance are related by the expression, $\varepsilon = 1 - \rho$, for opaque objects in the absence of specular reflections, so these parameters can be treated together. Penetration depth is a measure of the surface "thickness" of the object. The term transmittance is used in reference to the fraction of incident radiation which penetrates windows which are placed in front of the object to be measured.

Penetration Depth

If a sample is not at a uniform temperature, and, say, the bulk is warmer than the surface, then a fraction of the radiation emitted from the bulk of the material may be transmitted to the surface, enter the thermographic camera and cause an error in the temperature reading associated with the surface. The depth from which 1/e of the emitted thermal radiation reaches the surface is referred to as the penetration depth. Figure 4 shows an experiment set up to determine maximum limits of the surface "thickness." A thin sample of the material was placed in front of a hot source and was viewed with a thermographic camera. Muscle phantom material (9) was placed between two thin sheets of polyethylene; the thickness was established by a spacer in the sample holder. (For the case of bone, the holder consisted merely of a clamp.) The temperature of the sample was that of the ambient air. The signal detected by the camera was compared for the case when the hot source was behind the sample to that after it was removed. No difference was seen for sample thicknesses down to as small as 0.5 mm, the thinnest that we measured. Thus, the temperature measured with the thermographic camera is that of the surface and is not affected by thermal gradients in the bulk.

The Transmittance of Polyethylene

Muscle phantom material (9) is made from Silly Stuff (a gelling agent), polyethylene powder, salt and water and has a texture much like a sticky dough. It is highly viscous but does flow to take the form of its container, eventually. It is wet to the touch and dries when exposed to air. The loss of constituent water by evaporation changes the dielectric properties of the material. Furthermore, evaporation cools the surface especially in warmer regions of the phantom, thus depressing temperature variations. Thus, some sort of window must be placed over the surface to contain the material and to prevent evaporation of the water. The transmittance of the window then must be determined in order to compensate for the infrared radiation absorbed within it. Calculations of these effects are very difficult (10,11) because of the multiple reflections within the window and because of the radiation emitted by the window material. Nevertheless, practical experiments can be performed to evaluate the effective transmittance especially if the transmittance is high ($\tau \approx 0.9$)(12).

One experimental arrangement is shown in figure 5A. Two reference sources were used. The lower half of each was covered by polyethylene; the upper half was left bare. One of the sources was controlled at a temperature T_1 above ambient; the other source was allowed to equilibrate with ambient temperatures. Two thermograms were taken of the temperature difference between T_1 and T_2. The first, indicated by line (1), was taken just above the interface and the second (line 2) was taken just below the interface. Figure 5B is a multiple exposure of these thermograms. Actually, four exposures were taken by aiming the camera first at T_1 and then at T_2 for line 1 and then at T_1 and T_2 for line 2. In this way, all temperatures were recorded in the center of the field and errors due to

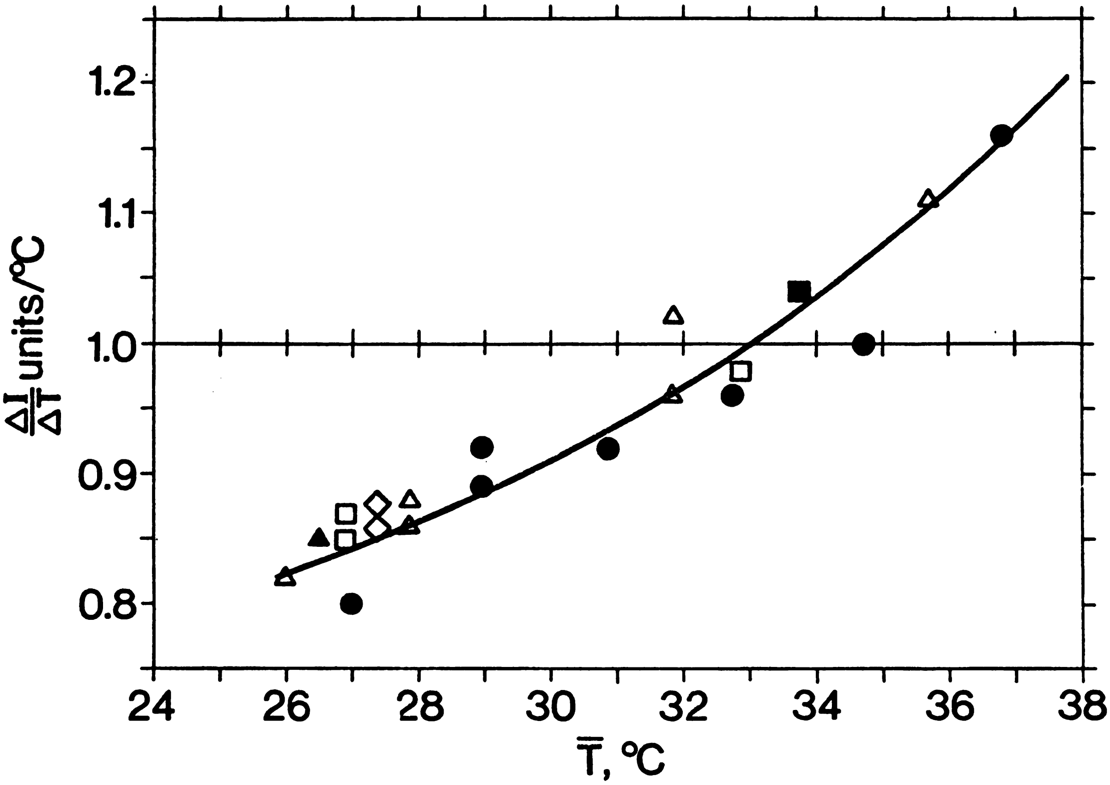


Figure 3. Calibration data for AGA thermovision located at BRH, Rockville, Maryland. Ten degrees fov lens, f/1.8, $\Delta I/\Delta T$ is the camera response to a temperature difference ΔT between two black sources. T is the "average temperature" of the two sources $(T_1 + T_2)/2$. ●, $\Delta T = 2^\circ$ C; ▲, $\Delta T = 3^\circ$ C; △, $\Delta T = 4^\circ$ C; ◇, $\Delta T = 5^\circ$ C; □, $\Delta T = 6^\circ$C; ■, $\Delta T - 8^\circ$ C.

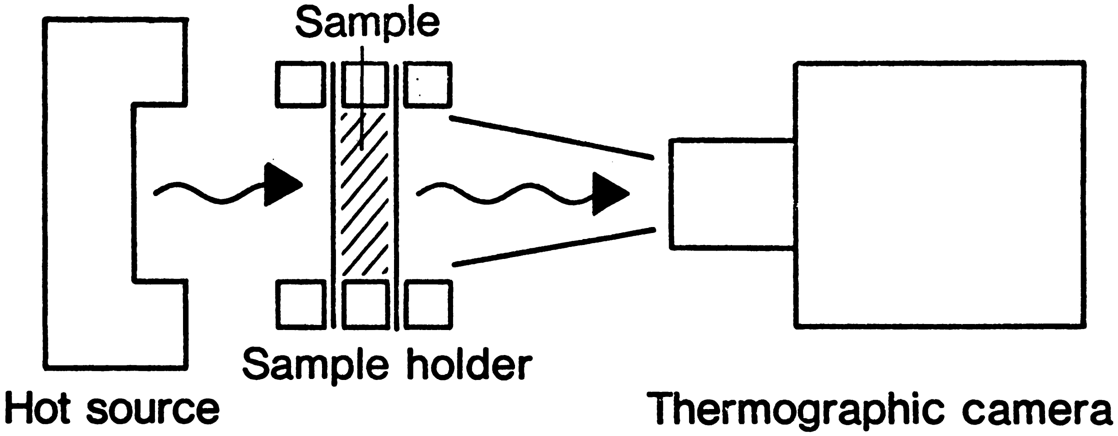


Figure 4. Sketch of the configuration used to test the infrared penetration depth of phantom materials. For muscle phantoms, the sample was sandwiched between two polyethylene (0.05 mm thick) windows. The thickness was determined by the center spacer in the holder.

lens aberration were avoided (see Fig. 2). Figure 5C is a sketch which interprets the thermogram. It is fairly straightforward to show that the transmittance of polyethylene window, τ_p is given by

$$\tau_p = \frac{(\Delta i_{12})\ \text{w/poly}}{(\Delta i_{12})\ \text{w/o poly}} \qquad (3)$$

One rather interesting feature is that the observations of T_2 (ambient temperature) give the same representation on the thermographic camera regardless of whether the polyethylene film is present. In other words, it records ambient temperature. This suggests a second method of measurement of τ_p, by looking at the small difference (at T_1) with more sensitivity. Figure 6 shows an experimental arrangement where this has been done. Half of the commercial black source was covered with the polyethylene material (0.05 mm). The difference in signal (Δi) on the thermographic camera between that with and that without the window material was measured. Room temperature was recorded as well with an additional mercury-in-glass thermometer, although one could have obtained this by use of the thermographic camera and the reference source. The equation relating these measurements to the transmittance is:

$$\tau_p = 1 - \frac{(\Delta i)}{I(T_1) - I(T_a)} \qquad (4)$$

$I(T_1) - I(T_a)$ is found through use of figure 3. These measurements are more precise than the first set because the small differences are determined with a more sensitive scale on the thermographic camera. The results of the transmittance measurments are shown in figure 7. The solid circles represent the data from Method 1, (that is Fig. 5) while the open circles and squares represent data taken by Method 2 (Fig. 6). Notice that the latter are more precise. The triangles, both open and closed, were extracted by a third method, similar to that shown in figure 4, but with the muscle phantom removed.

Emittance

Equation 2 shows that a temperature difference (or, $I(T_1) - I(T_2)$) on a surface is directly proportional to the emittance of that surface. Thus, knowledge of the emittance is necessary to obtain accurate temperature measurements. The direct approach would be to measure the amount of radiation emitted by the surface at a given temperature and compare that to the radiation of a blackbody source at the same temperature. However, it is very difficult to know the temperature of the surface. Measurements with a thermometer in the bulk of the material will give erroneous readings because of the steep thermal gradients near the surface of the object. Another approach, suggested to us by Mr. John Giest (13) of the National Bureau of Standards, is to compare the emittance of a surface to the emittance of a known material coating that surface. This is illustrated in figure 8A. The object is placed in an insulated and temperature controlled oven. Thermistors Th_1 and Th_2 measure block temperatures. They do not measure the surface temperature. Half of the object surface was coated with 3M Nextal paint. The reflectance, ρ, of 3M paint has been found to be 0.0275 (14). Since the emittance, ε, is equal to $1 - \rho$, $\varepsilon = 0.975$. When the sample is brought to a given temperature and observed with a thermographic camera, the temperature line profile will appear as in figure 8B. While temperature gradients exist near the edge of the object, in the central region, at the interface, the temperature is uniform. The sharp step represents the difference in emittance between that of the bone phantom surface and that of the 3M paint

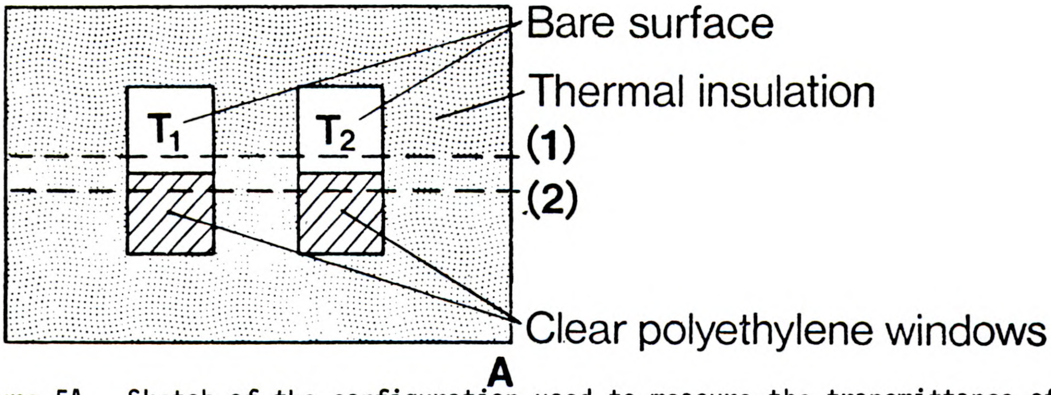

Bare surface
Thermal insulation
T_1
T_2
(1)
(2)
Clear polyethylene windows
A

Figure 5A. Sketch of the configuration used to measure the transmittance of polyethlene and hence the error introduced by its use as a window.

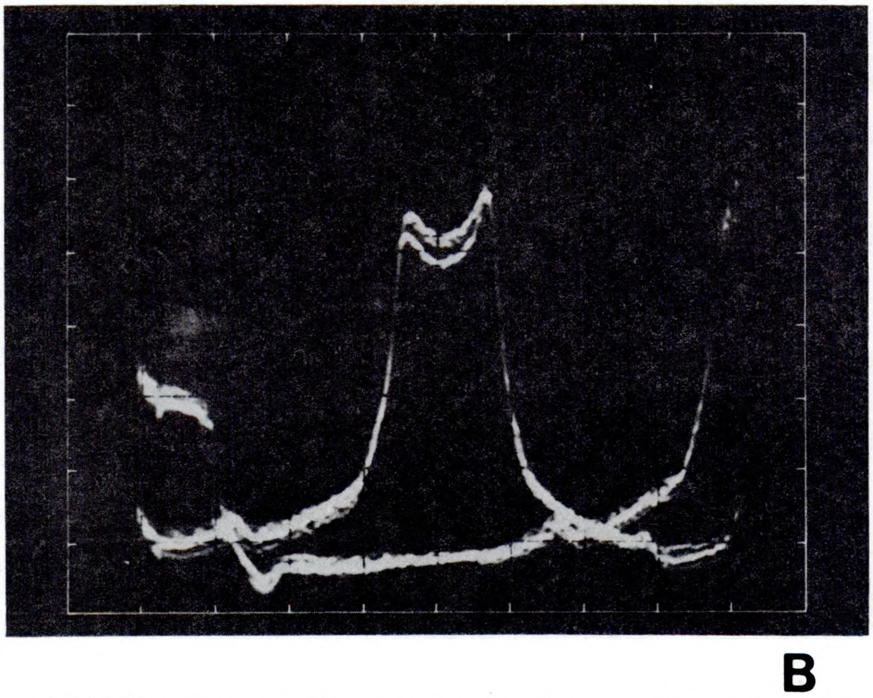


Figure 5B. Multiple-exposure thermograms showing raw data. The termographic camera was aimed so that the subject of interest appeared in the center of the field for each measurement in order to avoid errors associated with lens aberration (see figure 2).

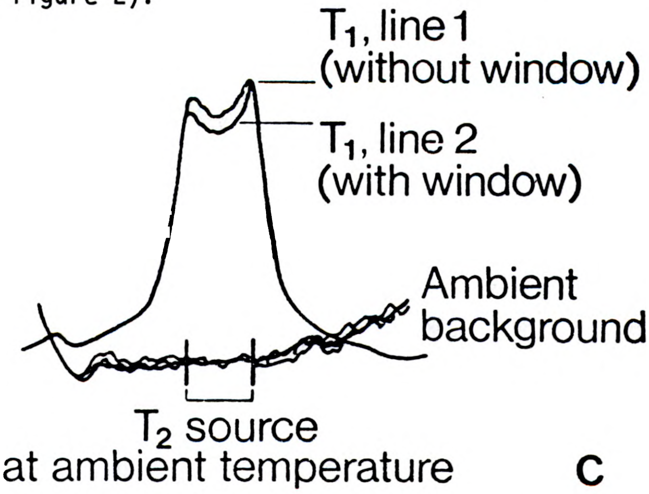


Figure 5C. Interpretive sketch of B.

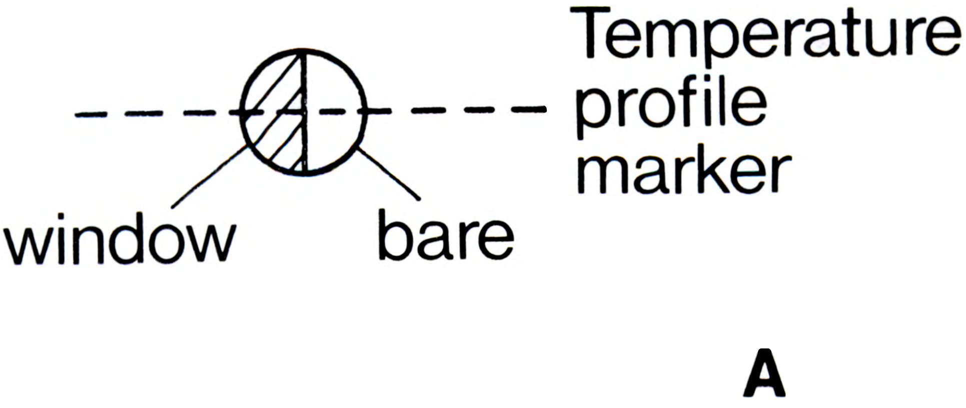

Temperature profile marker
window
bare
A

Figure 6A. Sketch of second method of measuring the transmittance of polyethylene.

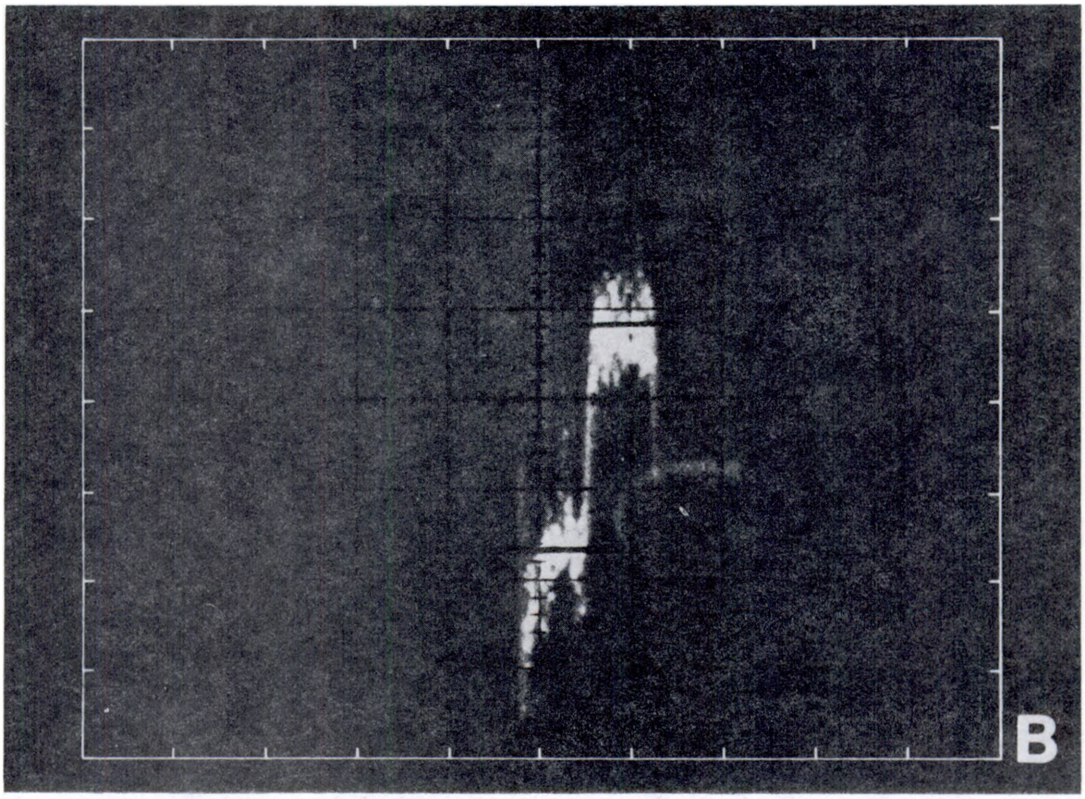


Figure 6B. Thermogram of typical data.

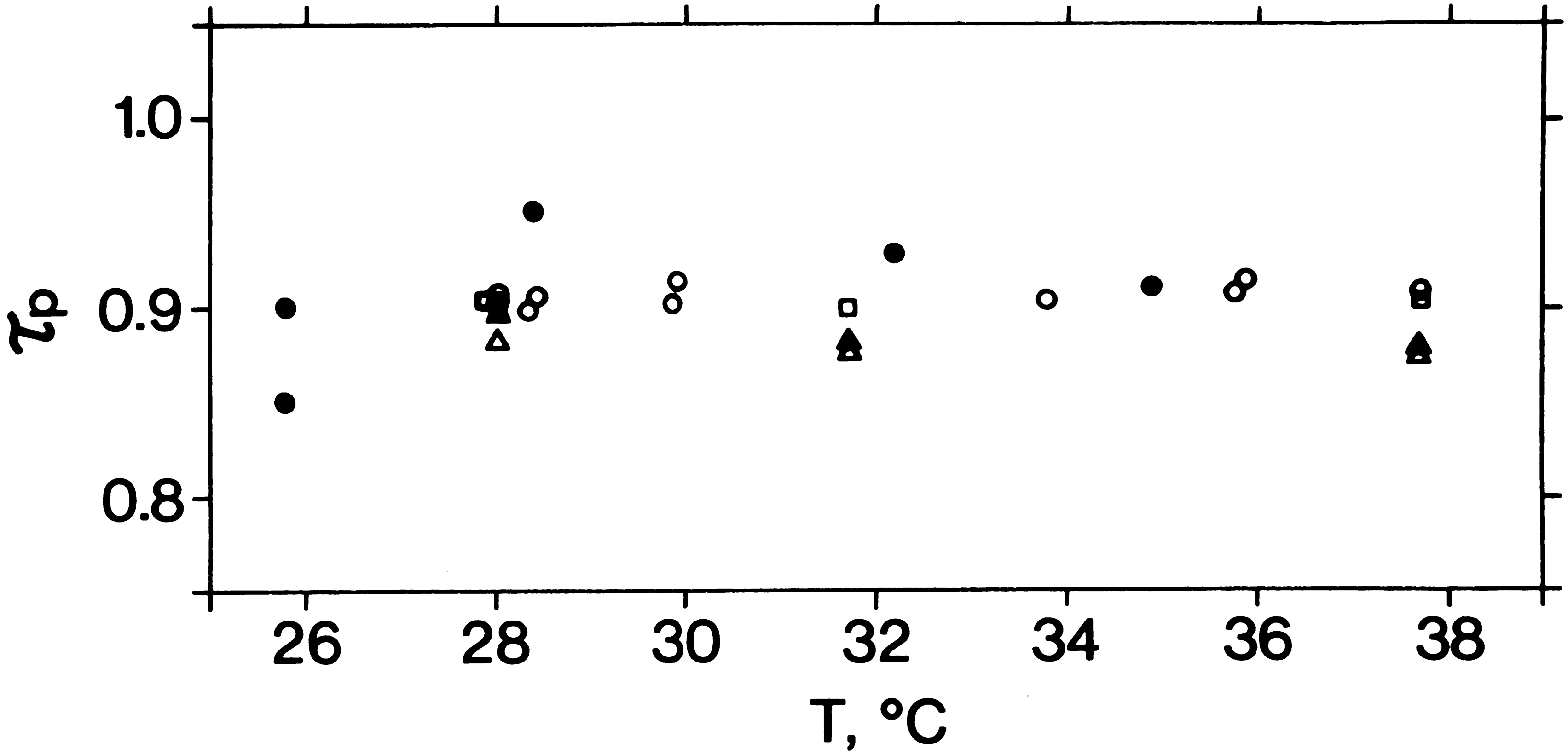
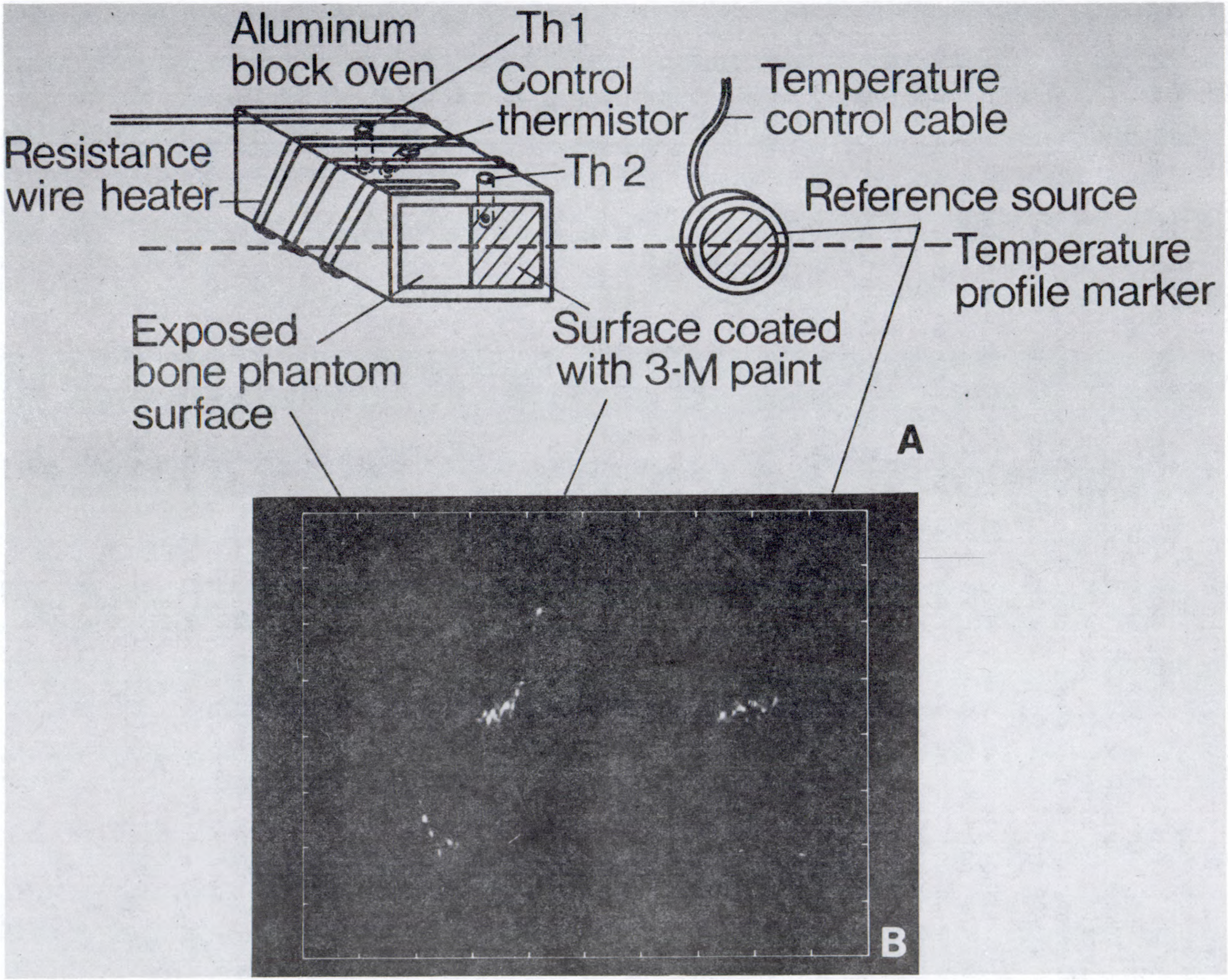


Figure 7. Transmittance of a sheet of 0.05 mm thick polyethylene as a function of source temperature. ●, data from first method (see figure 7); 0, data from second method (see figure 9); □, data from second method, taken much later than 0 data; Δ, ▲, data taken according to third method (see text and figure 6).


Aluminum block oven
Th 1
Control thermistor
Temperature control cable
Resistance wire heater
Th 2
Reference source
Temperature profile marker
Exposed bone phantom surface
Surface coated with 3-M paint
A
B

Figures 8A and 8B. (A) Sketch of method used to measure the emittance of an unknown material by comparison with that of a known material (3M paint). A temperature reference source is included as well. (B) Thermogram of typical data.

coating. The emittance of the bone can be calculated from this difference through use of equation 2, where $I(T_1) = I(T_2)$,

$$\varepsilon_2 = -\frac{\Delta i_{12}}{I(T_1) - I(T_a)} + \varepsilon_1 \qquad (5)$$

The subscripts 1, 2, and a, refer to reference material, unknown material and ambient. The temperature of the surface can be determined by comparison of the black reference source to the black 3M paint. $I(T_1) - I(T_a)$ can be determined directly with a second thermographic measurement. The results of these measurements are shown in figure 9 where the emittance was measured as a function of the temperature of the block. As expected, the precision is better at higher temperatures farther from ambient temperature. The various symbols refer to different experimental runs. For one set of measurements, a polyethylene window was placed over the surfaces and the results agree with the others.

Similar experiments were performed for muscle phantom material. In this case, the polyethylene film had to be placed over the material to prevent evaporation on constituent water from the surface. Half of the muscle surface was painted with 3M paint and was allowed to dry. The unpainted portion of the muscle surface dried also and was replaced with fresh muscle. The entire surface was covered with polyethylene. An experiment similar to that shown in figure 8 was performed. No discernable difference could be detected between the muscle phantom emittance and that of the 3M paint. The emittance of muscle is taken to be 0.908 or greater. Confidence in this result is strengthened by the fact that muscle has a wet texture and water has been shown (14) to have an emittance of 0.98.

Various other tests were performed. 3M paint was compared to acetylene soot and no difference was found. The emittance of bone material was compared directly with that of muscle by filling a hole in bone phantom with muscle material, placing polyethylene film over the two and carrying out the experiment as before. If muscle is taken as the standard ($\varepsilon_m = 0.98$) the emittance of bone is computed to be 0.88, which is 9 percent greater than the earlier figure of 0.81. Probably water vapor had spread over the bone from the muscle and had given it a higher effective emittance. This does, in fact, point out a very real problem for accurate temperature measurements. If the surface of the bone phantom could be coated with a black material (ε=1), errors associated with its low emittance value would be eliminated. Nevertheless, materials which are absorptive to radio frequency electromagnetic radiation cannot be used (such as water or 3M paint) because they alter the properties for which the phantom was designed initially.

TEMPERATURE MEASUREMENTS FOR THE THERMOGRAPHIC CAMERA

Having performed the above calibration, emittance measurements and transmittance and trasmittance measurements, and having verified that the camera in fact monitors temperatures of the surfaces of the material, quantitative thermometry can now be done. Rewriting equation (2) as:

$$I(T_2) - I(T_1) = -\frac{\Delta i_{12}}{\varepsilon_2} + \frac{\varepsilon_1 - \varepsilon_2}{\varepsilon_2}[I(T_1) - I(T_a)] \qquad (6)$$

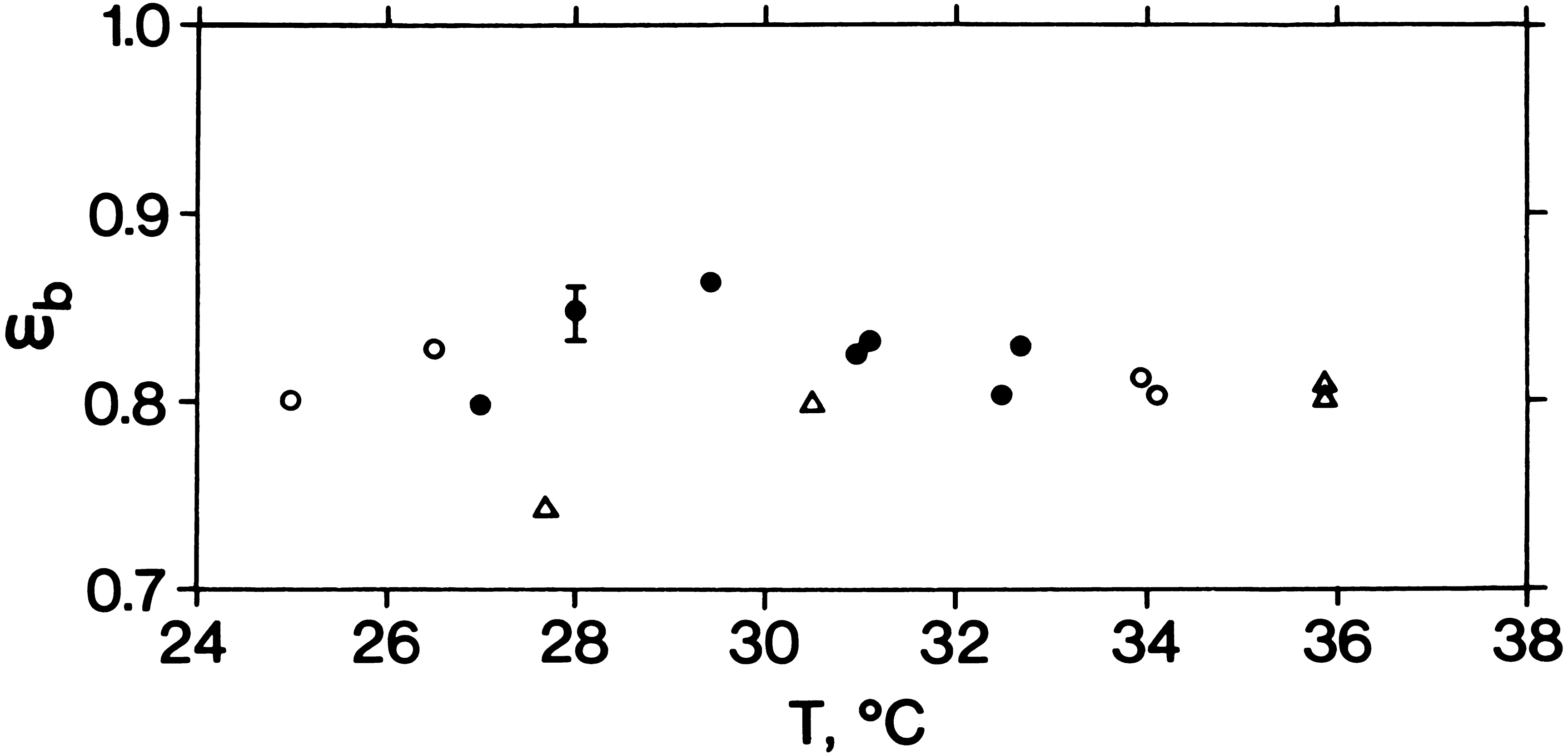


Figure 9. Emittance of bone phantom material as a function of temperature.
●, 0, taken on consecutive days;△, taken 6 months later.

Where 1, 2 and a refer, respectively to the reference source, the object with an unknown temperature and to ambient. The temperature T_2 is computed from the calibration of the camera response (Fig. 3) according to:

$$T_2 - T_1 = \frac{I(T_1) - I(T_2)}{\left(\frac{dI}{dT}\right)\ \bar{T}} \qquad (7)$$

Where $\bar{T}$ refers to the mean of T_1 and T_2, $(T_1 + T_2)^2$. All the terms in equations (6) and (7) have either been measured or have been determined previously. To illustrate this technique, planar electromagnetic phantoms (Fig. 10) were heated with a dielectric-loaded waveguide applicator at 2.45 GHz. Temperatures were monitored with a minimally perturbing thermistor probe (15), which uses a thermistor mounted on a sapphire needle and connected to high resistance leads which have been evaporated onto the needle. The bulk of the thermometer including conventional leads joining to the high resistance leads are shielded from the elctromagnetic field by the phantom tissue itself. The temperature rise measured on the thermometer was 3.35° C. Measured thermographically, (T_i = 23.4° C):

$$T_f - T_i = \frac{i_{12}}{\left(\frac{dI}{dT}\right)\bar{T}\ (\tau_p)} = \frac{(3.0\pm3 \text{ div})\ 1.6^{\circ}/\text{div})}{(.80)\ (.90)} = 3.3\pm0.3^{\circ}\text{C} \qquad (8)$$

Under the circumstances, the agreement is good. The measurement of bone phantom temperatures would require that a factor of $1/\varepsilon_b$ be included as well since $1/\varepsilon_b = 0.8$ (or perhaps 0.85 because of water from the muscle material).

SUMMARY

Quantitative thermometry with a thermographic camera requires familiarity with the characteristics of the camera including the temperature equivalent noise level, sources of errors such as lens aberrations, and the calibration of the specific camera. Furthermore, the radiation characteristics of the object must be known. These would include the object's emittance, the penetration depth of infrared radiation in the object, and the nature of the surface of the object to be measured. Oil, films and dirt will affect the emittance and hence, alter the radiation characteristics. Consideration must be given to the effects of any intervening materials such as a polyethylene window or dirt on the optical components. Finally, care must be taken to avoid experimental configurations which could cause special problems. In particular, the subject must receive only uniform isotropic background radiation. Special heat sources that could illuminate the object such as incandescent lights, electronic instruments or even the observer himself must be avoided. Convective air currents can cause non uniform heating or cooling of the surface. Viewing the surface at an oblique angle will cause an apparent temperature error. Finally, familiarity with the characteristics of the instrument, assurance that the properties of the source are the same during the temperature measurement as during preliminary emittance measurements, and verification that the camera is in good operating condition are necessary for good thermometry.

ACKNOWLEDGEMENTS

One of us (TCC) is indebted to the staff of the Electromagnetics Branch, Division of Electronic Products, Bureau of Radiological Health for their hospitality during two visits in which the experimental work was performed. Discussions with Mr. J. C. Richmond and with Mr. J. Geist of the National Bureau

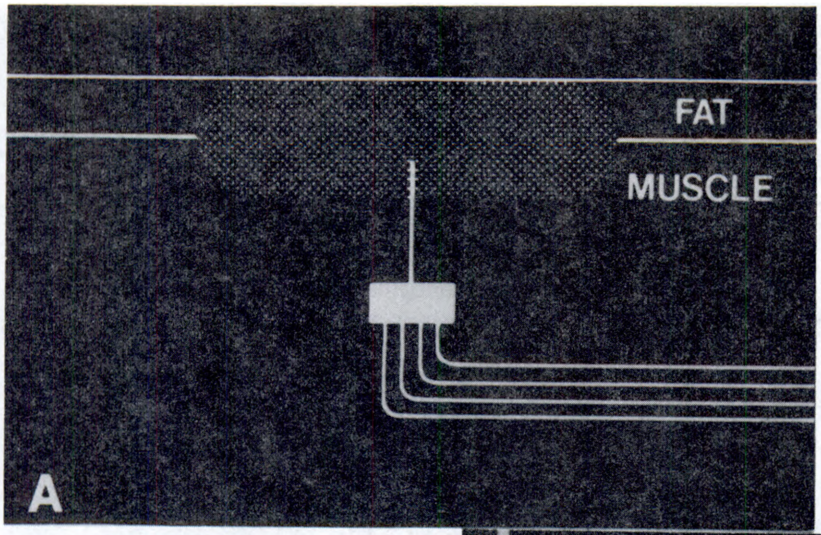
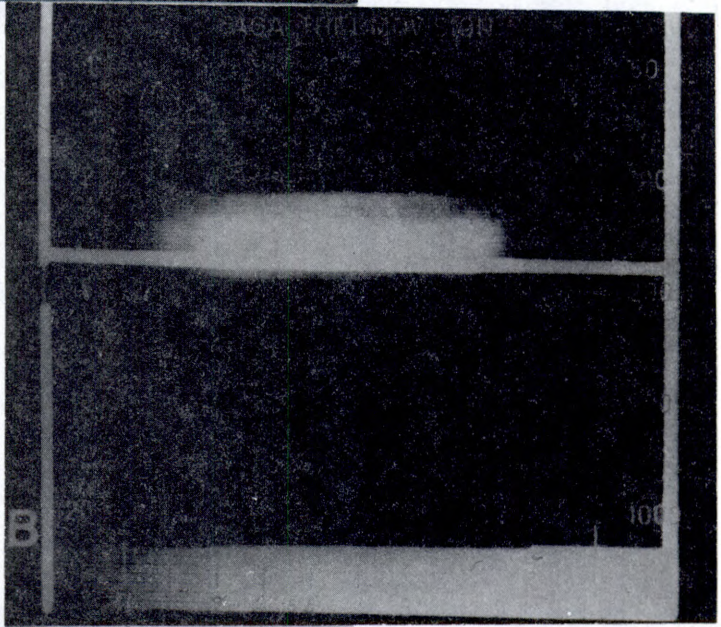


Figure 10. Comparative measurement of the temperature rise induced by microwave diathermy applicator using thermography and a minimally perturbing probe thermometer.

Figure 10A. Sketch of phantom model showing thermometer location.

Figure 10B. Two dimensional gray scale scan of heating pattern.



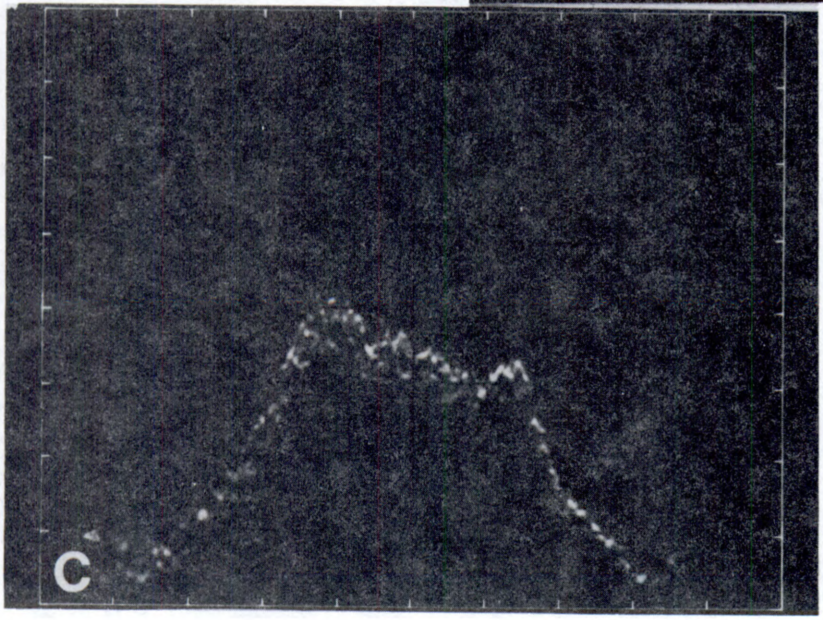


Figure 10C. Temperature profile through region sampled by thermometer probe. Care was taken to ensure that the probe was read at the instant the thermogram was taken.

of Standards were very helpful. Partial support was provided under DHEW contract, FDA-03189-76-J and by NCI grant CA 17343-01.

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OPEN DISCUSSION

DR.GUY: I am glad that somebody went through all this care to measure the emissivity and so forth. We did some crude experiments some years ago. Is your thermograph the industrial model or the medical model?

DR. KANTOR: It is the Industrial Model, 600.

DR. GUY: There is supposed to be a better resolution on a medical model for some reason; I do not know why. However, the specs show a little better resolution; that is the reason I was wondering about the 1° when you were indicating the noise. The other question is, the polyethylene film you used, is that the scotch tape material?

DR. KANTOR: No, this is not the scotch tape. It is commercial polyethylene film; the thickness is 0.05 mm.

DR. GUY: Did you ever make any measurements to differentiate between what might be going through the film by diffusion, as compared to being transmitted through?

DR. KANTOR: You mean heat diffusion?

DR. GUY: Yes, we have always wondered; we really have not done any measurements on this. What was the major mode of transfer through the film? Was this diffusion from the heating pattern underneath or the actual transmission of the infrared.

DR. KANTOR: I would like to say here that I am confident that Tom Cetas could answer this question; unfortunately, I cannot because I have not been warned about that aspect of the problem. However, I will talk to Tom over the phone and ask him about that. If I understand you correctly, you are worried about whether there is any diffusion of heat through the film.

Note added in proof (T. CETAS): The sum of the transmittance, emittance and reflectance equals unity for a given surface by the standard laws of radiometry. Since the transmittance is 0.9, the sum of the other two terms can only be 0.1. In other words, the dominant effect is transmission of the infrared rather than heat conduction to the film and subsequent emission from the polyethylene surface.

A FINITE ELEMENT TECHNIQUE FOR CALCULATING INDUCED INTERNAL FIELDS AND POWER DEPOSITION IN BIOLOGICAL MEDIA OF COMPLEX IRREGULAR GEOMETRY EXPOSED TO PLANE WAVE ELECTROMAGNETIC RADIATION

Stanley M. Neuder
Division of Electronic Products
Bureau of Radiological Health, FDA

ABSTRACT

A mathematical strategy is described by which induced electromagnetic fields and absorbed power density distributions may be calculated in biological media of complex irregular geometry exposed to plane wave RF and microwave radiation. Computer programs have been developed and utilized for calculating and graphing externally scattered and internally induced microwave fields in biological tissue. Results of calculations on a spherical multilayered model of the head and an irregularly shaped multilayered thigh model will be shown.

INTRODUCTION

In recent years, a number of researchers in the field of dosimetry and electromagnetic scattering have used computer programs to determine the induced fields and power deposition in biological media exposed to microwave and RF radiation. In this frequency range, biological tissue is mathematically simulated as "lossy" dielectric media, characterized by a finite electrical conductivity σ and dielectric constant K. The biological system being irradiated is usually idealized as either single and multilayered concentric spheres or single and multilayered concentric cylinders infinite in extent along the longitudinal axis. These geometrical idealizations allow for an exact mathematical solution of Maxwell's equations.

A model of this kind is shown in figure 1 in which a head is simulated by a central spherical core and five concentric spherical layers (1). These six regions, starting from the center, represent (a) brain, (b) cerebrospinal fluid, (c) membrane, (d) bone, (e) fatty tissue, and (f) skin, respectively. Using an outer diameter of 6.6 cm and a 3 GHz plane wave propagating along the z-direction with linear polarization along x, the exterior-scattered fields were calculated in the region surrounding the head (2). The computer plot of the results is shown in figure 2. The induced internal electric field distribution for the same model was also calculated and is shown in figure 3, for four different planes of the head. One sees that there are induced fields in the layers around the head although the radiation is incident from one direction only. The distribution of absorbed power density (in W/m^3) is plotted in figure 4 using the same six region head model but with an outer diameter of 3.3 cm.

In complex geometries where the biological system is substantially irregularly shaped or of asymmetric internal structure, one cannot solve the governing mathematical equations without recourse to numerical approximation techniques. Because of the pertinent applications to the field of dosimetry and public health, the capability of numerically calculating absorbed power distribution in any biological system, regardless of shape and internal structure, is being developed.

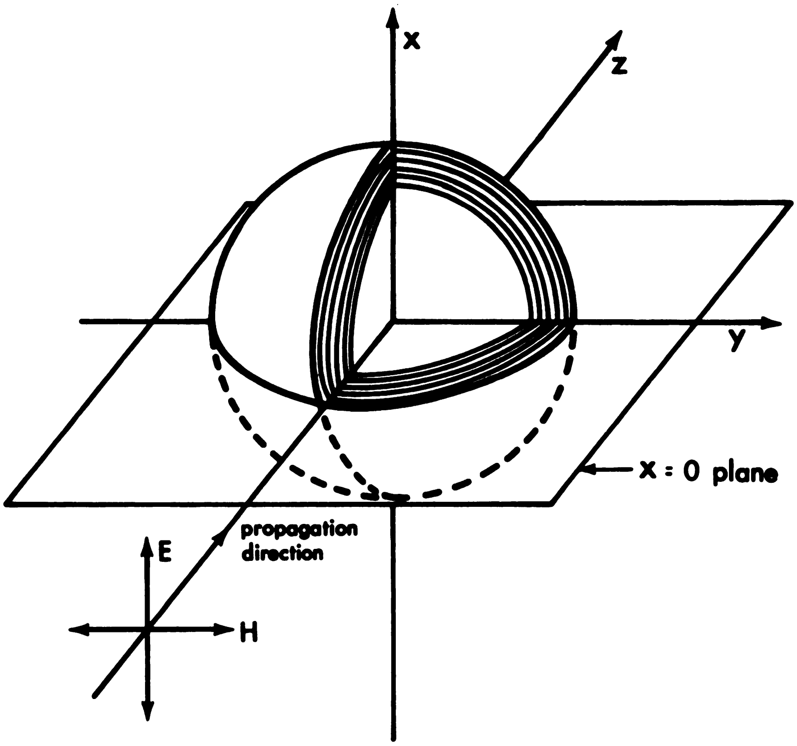
x
z
y
x = 0 plane
propagation direction
E
H

Figure 1. Spherical, multilayered model of the head.

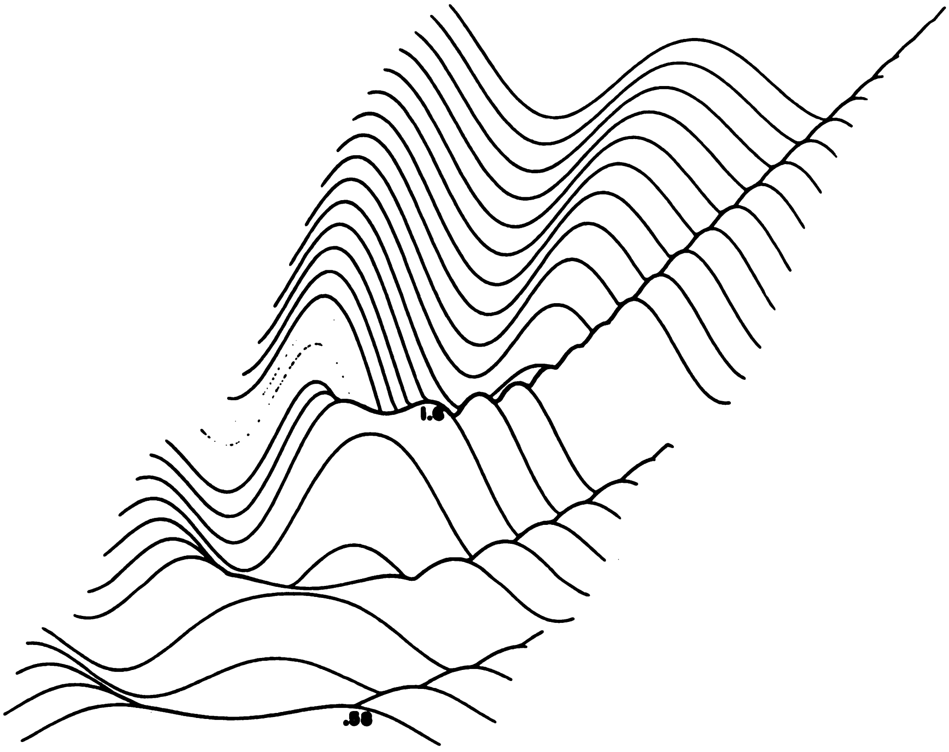

Figure 2. Three GHz electric field intensity scattered from a 6.6 cm diameter head (not shown) located in the trough near the center of the figure. Propagation is from lower left to upper right.

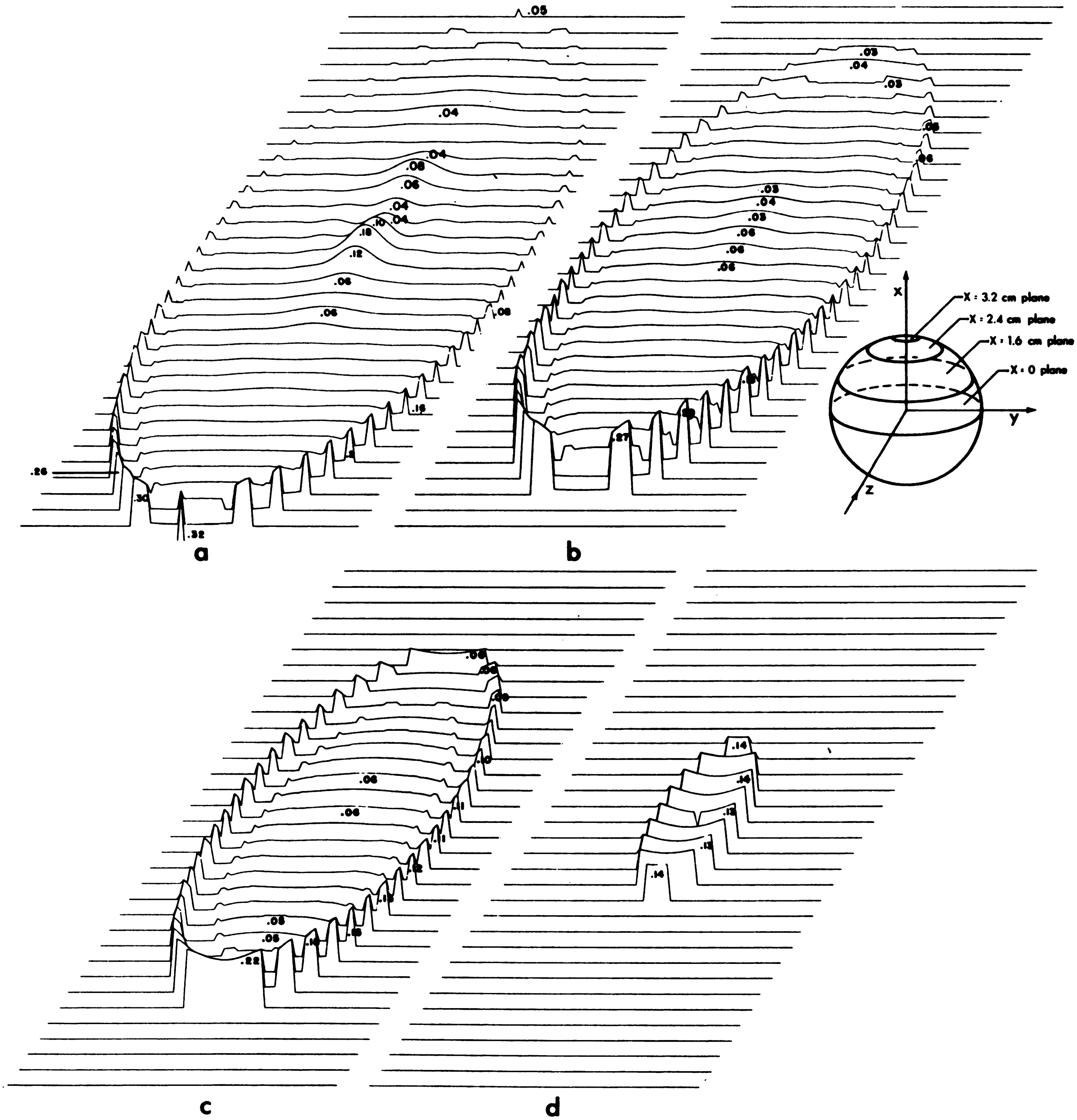

Figure 3. Induced internal electric field intensity in four planes of the head.

a. x = 0.0 cm plane
b. x = 1.6 cm plane
c. x = 2.4 cm plane
d. x = 3.2 cm plane

THEORETICAL DISCUSSION

What follows is an outline of the various mathematical strategies, with illustrative examples, used in developing a numerical technique for calculating induced internal fields in complex geometric structures.

The development begins with Maxwell's equations (3).

$$\nabla \times \vec{E} = -\mu \frac{\partial \vec{H}}{\partial t} \tag{1}$$

$$\nabla \times \vec{H} = \sigma \vec{E} + \varepsilon \frac{\partial \vec{E}}{\partial t} \tag{2}$$

$$\nabla \cdot \vec{E} = \rho/\varepsilon \tag{3}$$

$$\mu \nabla \cdot \vec{H} = 0 \tag{4}$$

These partial differential equations govern the behavior of the electromagnetic fields in biological media. The medium is mathematically characterized by the electrical conductivity σ, the electrical permittivity ε, and the magnetic permeability μ. From Maxwell's equation we derive the wave equation governing the field vector E and H in a source-free medium (charge density, p = o).

$$\nabla^2 \vec{E} - \mu\varepsilon \frac{\partial^2 \vec{E}}{\partial t^2} - \mu\sigma \frac{\partial \vec{E}}{\partial t} = 0 \tag{5}$$

For plane wave irradiation of circular frequency $w = 2\pi f$, the fields are time harmonic of the form

$$\vec{E}(x, y, z, t) = \vec{E}(x, y, z)\, e^{-i\omega t} \tag{6}$$

Substituting (6) into (5) yields the Helmholtz equation

$$\nabla^2 \vec{E} + k^2 \vec{E} = 0 \tag{7}$$

where

$$k^2 = \mu\varepsilon\omega^2 + i\mu\sigma\omega \tag{8}$$

The propagation constant, k, is complex in a finite conducting (lossy dielectric) medium. Thus, the time harmonic fields are governed by the Helmholtz equation.

The development continues with the calculus of variations (4). In the calculus of variations, the function is sought that renders the functional stationary. Consider the functional I[y(x)] given by equation 9

$$I[y(x)] = \int_{x_1}^{x_2} f[x, y(x), y'(x)]\, dx \tag{9}$$

The value of the functional depends on the function y(x) in the integrand. For example, the distance between two points, x_1 and x_2, in a plane is given by the functional

$$I(y) = \int_{x_1}^{x_2} \sqrt{1 + (^{dy}/_{dx})^2}\; dx \tag{10}$$

This distance depends on the path chosen to connect the points. Thus, the value of the functional depends on the function y(x). Of all the admissible functions that satisfy the boundary conditions (i.e., passing thru the end points), one will render the integral I(y) an extremum. This one particular function is found by requiring that the integrand $f(x,y,y^1)$ of the functional I(y), satisfy the associated Euler equation

$$\frac{d}{dx}\left(\frac{\partial f}{\partial y'}\right) - \left(\frac{\partial f}{\partial y}\right) = 0 \tag{11}$$

For the distance between two points in a plane, $f = (1 + y^2)^{\frac{1}{2}}$ from equation 10. Substituting into the Euler equation yields y^1 = a constant. A simple integration of this results in the form

$$y = ax + b \tag{12}$$

which represents a straight line. This simply says that the function that represents a straight line is the one that minimizes the functional. (or, the shortest distance between two points in a plane is a straight line). The associated Euler equation of a functional is obtained by expressing the dependent variables in the integrand as functions of a variational parameter and differentiating with respect to this parameter in order to locate the extremum. Thus, the functional in equation 9 is written as

$$\frac{\partial}{\partial\alpha}\int_{x_1}^{x_2} f[x, y(\alpha), y'(\alpha)]\, dx = 0 \tag{13}$$

in order to obtain the associated Euler equation. A functional that is applicable to the electromagnetic field determination in a region of volume V bounded by surface S is

$$I = \int f\left(x, y, z, \phi, \frac{\partial\phi}{\partial x}, \frac{\partial\phi}{\partial y}, \frac{\partial\phi}{\partial z}\right) dV + \int (\alpha\phi + \beta\phi^2) dS \tag{14}$$

where $\phi = \phi(x,y,z)$ represents the class of functions that satisfy the boundary conditions of the problem. The function ϕ may represent one of the three components of the electric or magnetic field.

Performing the variational operation, $\delta I = 0$, one obtains two associated Euler equations given by

$$\frac{\partial f}{\partial\phi} - \frac{\partial}{\partial x}\left(\frac{\partial f}{\partial\phi_x}\right) - \frac{\partial}{\partial y}\left(\frac{\partial f}{\partial\phi_y}\right) + \frac{\partial}{\partial z}\left(\frac{\partial f}{\partial\phi_z}\right) = 0, \qquad \text{in V} \tag{15}$$

$$\ell_x\left(\frac{\partial f}{\partial\phi_x}\right) + \ell_y\left(\frac{\partial f}{\partial\phi_y}\right) + \ell_z\left(\frac{\partial f}{\partial\phi_z}\right) + \alpha + \beta\phi = 0, \qquad \text{on S} \tag{16}$$

where ϕ_x, ϕ_y, ϕ_z represent partial differentiation with respect to x, y, and z, respectively, and ℓ_x, ℓ_y, ℓ_z, are direction cosines of the normal to the outer surface with the x, y, z axes.

The function $\phi(x,y,z)$ that satisfies these two Euler equations will be the one that minimizes the integral I, given in equation 14. If the integrand of equation 14 is of the form

$$f = |\nabla\phi|^2 - k^2\phi^2 \tag{17}$$

then, substitution into equation 15 yields the Helmholtz equation

$$\nabla^2\phi + k^2\phi = 0 \tag{18}$$

Thus, the function ϕ that makes the functional (equation 14) an extremum is also the one that is the solution to the Helmholtz equation. Thus, instead of trying to solve the Helmholtz partial differential equation directly, one may equivalently obtain the solution of the Helmholtz equation by a variational approach. Simply stated, by finding the function ϕ that minimizes the integral I, one has simultaneously found the solution ϕ to the Helmholtz equation.

A more familiar example would be that of finding the field distribution in problems of electrostatics or thermal conduction. These problems are described by Laplace's equation

$$\nabla^2\phi = 0 \tag{19}$$

in a domain D, with Dirichlet boundary conditions

$$\phi = g(x, y, z) \tag{20}$$

or with Cauchy boundary conditions

$$\frac{\partial\phi}{\partial n} + \alpha\phi + \beta = 0 \tag{21}$$

on surface S. The solution $\phi(x,y,z)$ of Laplace's equation that satisfies these boundary conditions may be obtained by solving the equivalent variational problem; namely, finding the function ϕ that minimizes the functional

$$I = \int|\nabla\phi|^2 dV + \int(\alpha\phi + \tfrac{1}{2}\beta\phi^2)dS \tag{22}$$

The minimization of this functional will yield the same ϕ that is the solution to the Laplace equation. Similar remarks apply to solving the Poisson equation in problems where sources are present or the biharmonic equation in problems where the medium is anisotropic and inhomogeneous.

The variational approach is now used as a vehicle for an approximation method for finding the induced fields in biological media. The approximation method is based on a frequently used technique in mathematics called the method of trial solutions or Ritz method (5,6). As applied here to the electromagnetic problem the Helmholtz partial differential equation is replaced by the equivalent functional $I(\phi)$ to be minimized. We, therefore, desire to satisfy

$$I\ (\phi) = \text{minimum} \tag{23}$$

in some domain with associated boundary conditions. The desired solution, ϕ, to the Helmholtz equation is known to give an extremum value to the functional I.(ϕ) An approximate or trial solution, designated as ϕ, of the form

$$\hat{\phi} = \sum_j C_j\ \phi_j \tag{24}$$

is substituted into the functional so that the problem becomes that of satisfying

$$I(\hat{\phi}) = \text{minimum} \tag{25}$$

The ϕ_j are linearly independent functions in the domain and the C_j are unknown parameters. The above technique is illustrated in the following example. We wish to find the solution to the differential equation

$$\frac{d^2y}{dx^2} + \frac{2}{x}\left(\frac{dy}{dx}\right) + y = 0 \tag{26}$$

in the one-dimensional domain x in (o,π) subject to boundary (end point) conditions

$$y(o) = 1, \quad y(\pi) = 0 \tag{27}$$

Instead of solving this differential equation directly, we replace it with the equivalent functional

$$I(y) = \int_o^{\pi} x^2\left\{\left(\frac{dy}{dx}\right)^2 - y^2(x)\right\} dx \tag{28}$$

The strategy here is to seek $y(x)$ from the functional representation since the $y(x)$ that minimizes the functional is the same $y(x)$ that satisfies the original differential equation. The method of trial solutions to is now used to find an approximation $\hat{y}(x)$ to the actual solution $y(x)$. As used in this example, the technique is called the Classical Ritz method. (Do not proceed to the associated Euler equation, for that would lead us back to the original differential equation.) Writing the trial solution in the form

$$\hat{y} = \left(1 - \frac{x}{\pi}\right)\sum_{n=o}^{\alpha} \alpha_n x^n \tag{29}$$

The coefficient insures that the boundary conditions are met. For simplicity use only the first two terms of the series; namely,

$$\hat{y} = \left(1 - \frac{x}{\pi}\right)(1 + \alpha x) \tag{30}$$

Substituting this into the functional (equation 28) we obtain

$$I(\alpha) = \int_o^{\pi}\left\{\left[\alpha^2 - \frac{2\alpha}{\pi} - 1\right] x^2 - \left[\frac{4}{\pi}\alpha^2 - \left(\frac{4}{\pi^2} - 2\right)\alpha - \frac{2}{\pi}\right] x^3 \right.$$

$$\left. - \left[\alpha^2 - \frac{4\alpha}{\pi}\right] x^4 + \left[\frac{2\alpha^2}{\pi} - \frac{2\alpha}{\pi}\right] x^5 - \left[\frac{\alpha^2}{\pi^2}\right] x^6\right\} dx \tag{31}$$

Calculating $(\partial I(\alpha)/\partial\alpha) = 0$ one obtains the value of α which minimizes the functional $I(\alpha)$. Substituting this value of α into the approximate solution (equation 30) one obtains

$$\hat{y}(x) = 1 - \frac{1}{3}x + \frac{5}{1000}x^2 \tag{32}$$

As the approximate solution of equation 26. The exact solution of equation 26 is known to be the spherical Bessel function

$$y(x) = \frac{\sin x}{x} \tag{33}$$

A comparison of these two solutions is given in table 1. It may be seen that y(x) and y(x) differ by no more than 25 percent in the domain of interest.

Table 1. Comparison of approximate solutions, using equation 32, with exact solutions, using equation 33

x	Approximate solution, $\hat{y}(x)$	Exact solution y(x)
0.2	.93	.99
0.4	.87	.97
0.6	.80	.94
0.8	.74	.90
1.0	.67	.84
1.2	.61	.78
1.4	.54	.70
1.6	.48	.62
1.8	.42	.54
2.0	.35	.45
2.2	.29	.37
2.4	.23	.28
2.6	.17	.20
2.8	.10	.12
3.0	.04	.05
π	.01	0

A closer approximation to the actual solution may be achieved by including more terms of the series expression for y; namely,

$$\hat{y} = \left(1 - \frac{x}{\pi}\right)\sum_{n=0}^{j} \alpha_n x^n \tag{34}$$

However, additional equations of the form

$$\frac{\partial I(\alpha_0, \alpha_1, . . . \alpha_j)}{\partial \alpha_i} = 0, \qquad i = 0, 1, 2, . . ., j \tag{35}$$

must be calculated. Equation 35 yields a system of linear, algebraic simultaneous equations in the α_j. This approximation technique reduces the problem of finding the solution of a differential equation to one of solving a system of simultaneous algebraic equations. The goal is then to find trial functions that are convenient for minimizing the functional and for computing the electromagnetic fields. In order to achieve both convenience and computability we resort to the finite element method, (FEM).

The underlying concept of finite elements is simple (5,6). One starts by subdividing the domain of interest into smaller regions called elements. These elements must be easy for the computer to record and identify. For a two-dimensional domain these elements are polygons. In three dimensions the elements are polyhedra. Within each element the trial function is then chosen to have a simple form, say bilinear in the variables or a 2nd or 3rd degree polynomial. In this way piecewise polynomials can approximate an unknown solution. Further advantages of subdividing a region into elements are that boundary conditions are easier to impose locally, for instance, along the edge of a triangle or rectangle rather than along a larger more complicated boundary. In addition, accuracy of approximation can be improved if desired, not by including more terms in the polynomial or by more complex trial functions but rather by refining the subdivisions while retaining the same simple polynomial form. The virtue of this refinement is that the associated computer program need not be altered to accomodate this change.

An illustration of the combined use of the FEM, trial functions, and variational calculus in one, two, and three-dimensional problems now follows. The first example is that of finding the solution of the differential equation

$$\frac{d^2y(x)}{dx^2} - y(x) = 0 \tag{36}$$

in the one-dimensional domain (0,2) subject to boundary (end-point) conditions

$$\begin{cases} y = 1 & \text{at} \quad x = 0 \\ y = e^2 & \text{at} \quad x = 2 \end{cases} \tag{37}$$

The equivalent functional to be minimized by some function y(x) would be

$$I = \int_0^2 \left\{ \left[\frac{d}{dx} y(x)\right]^2 + \left[y(x)\right]^2 \right\} dx \tag{38}$$

The unknown function y(x) that minimizes the functional will be the solution of the differential equation (equation 36). To find y(x), the domain (0,2) is arbitrarily subdivided into four elements, labeled m_1 through m_4. The end points of the elements are called nodes (Fig. 5). At each of the five nodes, the solution is designated by y, called nodal values, that are independent of x, and within each element we approximate the unknown exact solution y(x) by a trial solution y(x). Let us choose a trial solution of the form

$$\hat{y} = a + bx \tag{39}$$

There will be four such trial solutions, one in each element. The coefficients a and b in each y are determined by a linear interpolation between nodal values.

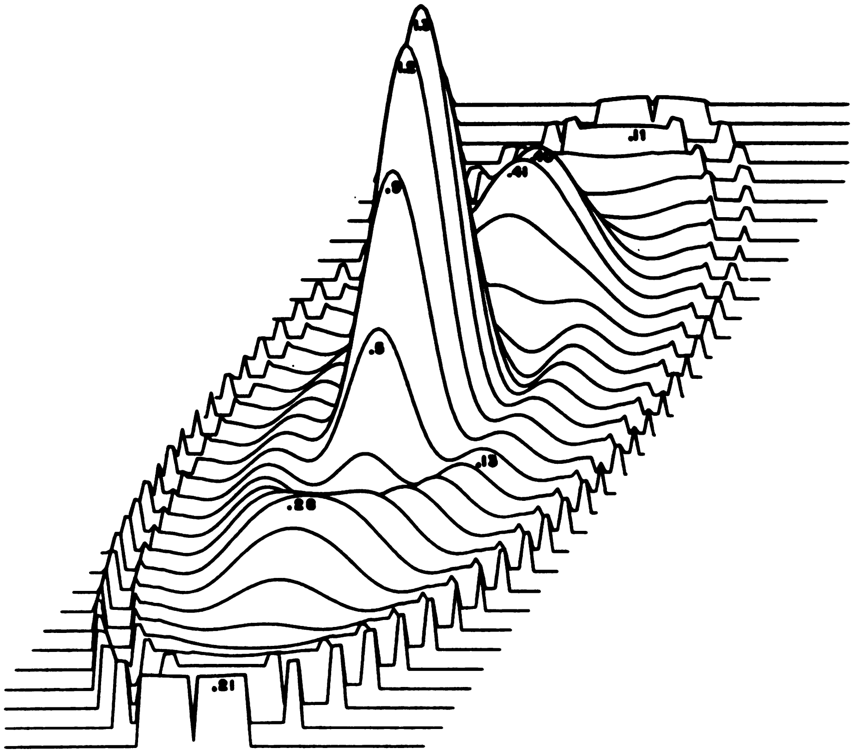

Figure 4. Absorbed power density distribution in a 3.3 cm diameter multilayered spherical head model.

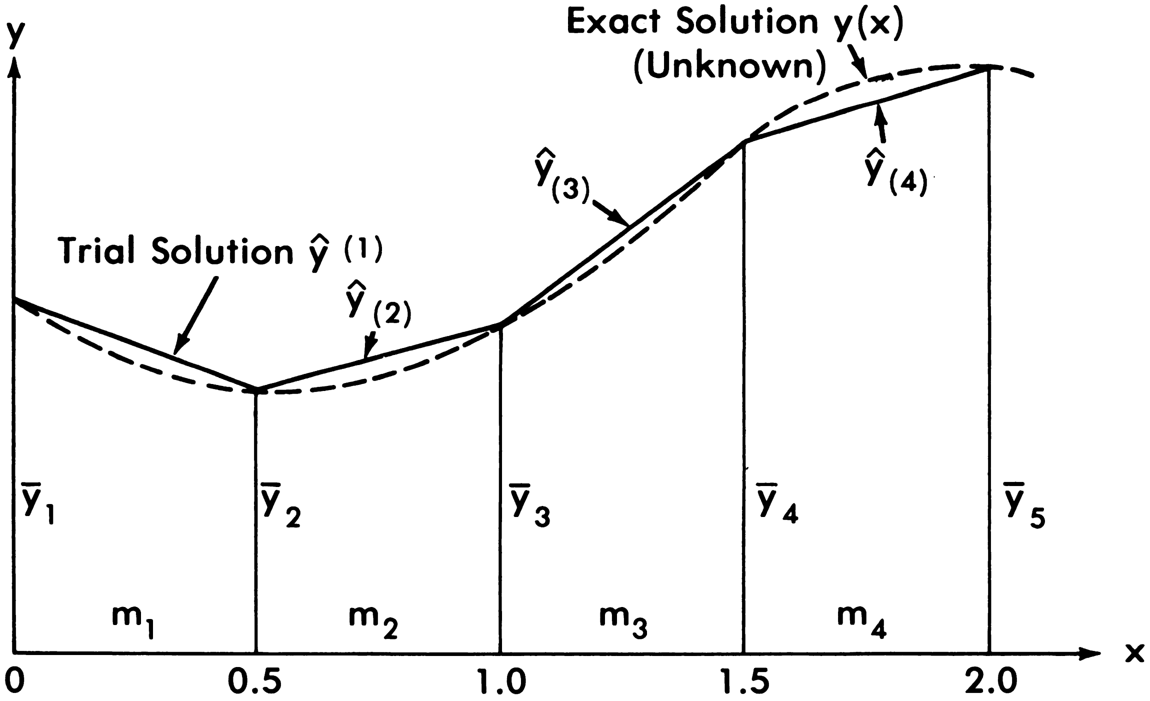
y
Exact Solution y(x)
(Unknown)
$\hat{y}_{(3)}$
$\hat{y}_{(4)}$
Trial Solution $\hat{y}^{(1)}$
$\hat{y}_{(2)}$
$\bar{y}_1$
$\bar{y}_2$
$\bar{y}_3$
$\bar{y}_4$
$\bar{y}_5$
m_1
m_2
m_3
m_4
x
0
0.5
1.0
1.5
2.0

Figure 5. Subdivision of a one-dimensional domain into four finite elements and associated linear trial solutions.

The graphic representation of the four trial solutions are the line segments, the y_i shown in figure 5 connecting the adjacent nodal values, y_i. From trigonometric considerations and using $\Delta x = 0.5$ in this example, the trial solution for the m-th element is

$$\hat{y}^{(m)} = (1-2x + 2x_{i-1})\bar{y}_{i-1} + 2(x - x_{i-1})\bar{y}_i \;, \qquad x \text{ in } m \tag{40}$$

Where y_{i-1} and y_i are the two nodal values at the left and right nodes of that element and x_{i-1} is the x-coordinate of the left node. The quantities in parenthesis, called shape functions, are functions of the independent variable x and the coordinates of the nodes. There are four such functions of the same form, each non-zero only within their respective elements so that the functional becomes the sum of four integrals

$$I = \sum_{m=1}^{4} \int_m \left\{ (\hat{y}^{(m)})^2 + \left(\frac{d}{dx} \hat{y}^{(m)} \right)^2 \right\} dx \tag{41}$$

Substituting the four corresponding $y^{(m)}$ into equation 41 and integrating, yields a twelve-term polynomial (3 from each finite element) of the form

$$I = \sum_{i=1}^{4} \left(\alpha_i \bar{y}_i^2 + \beta_i \bar{y}_i \bar{y}_{i+1} + \gamma_i \bar{y}_{i+1}^2 \right) \tag{42}$$

The 12 coefficients α_i, β_i and γ_i are constants that have been determined by the integration and the y's are unknowns. Further, $y_1 = 1$ and $y_5 = e^2$ from equation 37 so that I is reduced to a function of 3 unknowns; y_3, y_4 and y_5. For the functional in equation 42 to be minimized, require

$$\frac{\partial I}{\partial \bar{y}_i} = 0 \;, \qquad i = 2, 3, 4. \tag{43}$$

These three equations, together with the two boundary conditions result in a system of 5 simultaneous linear algebraic equations,

$$\left.\begin{aligned}
&\bar{y}_1 = 1.0 \\
&\bar{y}_1 - 2.261\bar{y}_2 + \bar{y}_3 = 0 \\
&\bar{y}_2 - 2.261\bar{y}_3 + \bar{y}_4 = 0 \\
&\bar{y}_3 - 2.261\bar{y}_4 + \bar{y}_5 = 0 \\
&\bar{y}_5 = 7.389
\end{aligned}\right\} \tag{44}$$

Solving simultaneously, one obtains the five nodal values that are approximations to the five exact solutions at the node positions. Shown in table 2 are the approximate solutions and the exact solutions at those points. The exact values are calculated from $y(x) = e^x$, which is the exact solution to the differential equation (equation 36).

Table 2. Comparison of approximate solutions using equation 44 with exact solutions using $y = e^x$

Node No.	x-coordinate	$\bar{y}$ approximate solution	y exact solution
1	0	1	1
2	.5	1.64	1.65
3	1.0	2.70	2.72
4	1.5	4.46	4.48
5	2.0	7.39	7.39

The problem is now considered solved in that approximate solutions have been obtained at the five desired points in the domain. Obtaining approximate solutions at other points requires a finer subdivision of the domain.

The five approximate (trial) solutions may now be obtained, if one so desires, from equation 40 and table 2. These are linear algebraic polynomials in x that describe the linear segments connecting the ordinates in figure 5.

For example, $\hat{y}^{(1)} = 1 + 1.28x$ will be the equation of the segment that approximates the true solution in the domain $o < x < 0.5$. (Fig. 5).

For two-dimensional problems (7), the domain is subdivided in the x-y plane into triangular finite elements. The m^th element will have three associated nodes (vertices) as shown in figure 6. The form of the trial solution for this element is chosen as

$$\hat{\phi}^{(m)}(x,y) = a + bx + cy \tag{45}$$

This represents a flat, triangular surface (analogous to the line segments in figure 5) approximating the true solution surface $\phi(x,y)$ over the m-th element. The three nodal functions for this element are

$$\bar{\Phi}_j = a + bx_j + cy_j \ , \qquad j = 1, 2, 3. \tag{46}$$

These are analogous to the nodal values $\bar{y}_i$ in figure 5.

In three-dimensional problems, the domain is subdivided into tetrahedral finite elements. For the m^{th} element the trial function is of the form

$$\hat{\phi}^{(m)}(x, y, z) = a + bx + cy + dz \tag{47}$$

and four nodal functions are of the form

$$\bar{\phi}_i = a + bx_i + cy_i + dz_i \ , \qquad i = 1, 2, 3, 4. \tag{48}$$

As in equation 40, for the one-dimensional case previously illustrated, the trial solution here becomes

$$\hat{\phi}^{(m)} = \frac{1}{\Delta_m}\Big[(g_{11} + g_{21}x + g_{31}y + g_{41}z)\ \bar{\Phi}_1 + (g_{12} + g_{22}x + g_{32}y + g_{42}Z)\ \bar{\Phi}_2 + (g_{13} + g_{23}x + g_{33}y + g_{43}z)\ \bar{\Phi}_3 + (g_{14} + g_{24}x + g_{34}y + g_{44}z)\Big]\ \bar{\Phi}_4 \qquad (49)$$

Where the g_{ij} are 3 X 3 determinants in the nodal coordinates of the m-th tetrahedron, $\Delta^{(m)}$ is a 4 X 4 determinant in the same nodal coordinates, and the parenthesis are the shape functions.

To illustrate this discussion, consider a lossy dielectric cube in an electromagnetic field. Subdivide the cube into five tetrahedra, (five finite elements and eight nodes), as shown in figure 7. The five tetrahedra fill the cubic space. They are also shown individually in the right half of the figure in their original orientation within the cube. Just as in the one-dimensional case this choice of subdivision will provide approximate field solutions only at the vertices of the cube, i.e., at the 8 nodes. To find these solutions, substitute equations 17 and 49 into equation 14. From the volume integral one obtains, in matrix form,

$$\int \left\{ |\nabla\phi|^2 - k^2\phi^2 \right\} dV = \sum_m \frac{1}{\Delta^2_m} \left\{\bar{\phi}\right\}^T \left[G_m\right]^T \left[M_m\right] \left[G_m\right] \left\{\bar{\phi}\right\} \qquad (50)$$

where the sum is over m tetrahedra, and where

$$\left\{\bar{\phi}\right\} = \begin{pmatrix} \bar{\phi}_1 \\ \bar{\phi}_2 \\ \bar{\phi}_3 \\ \bar{\phi}_4 \end{pmatrix} \qquad (51)$$

$$\left[G_m\right] = \begin{pmatrix} g_{11} & g_{12} & g_{13} & g_{14} \\ g_{21} & g_{22} & g_{23} & g_{24} \\ g_{31} & g_{32} & g_{33} & g_{34} \\ g_{41} & g_{42} & g_{43} & g_{44} \end{pmatrix}_m \qquad (52)$$

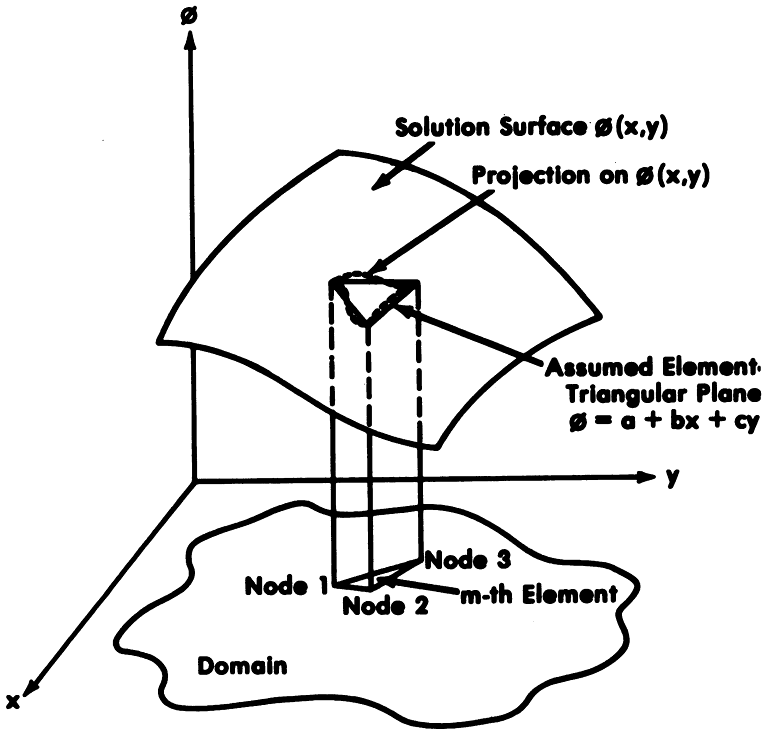
Ø
Solution Surface Ø(x,y)
Projection on Ø(x,y)
Assumed Element Triangular Plane
Ø = a + bx + cy
y
Node 3
Node 1
Node 2
m-th Element
Domain
x

Figure 6. Subdivision of a two-dimensional domain (in the x-y plane) into triangular finite elements and associated plane triangular trial solutions.

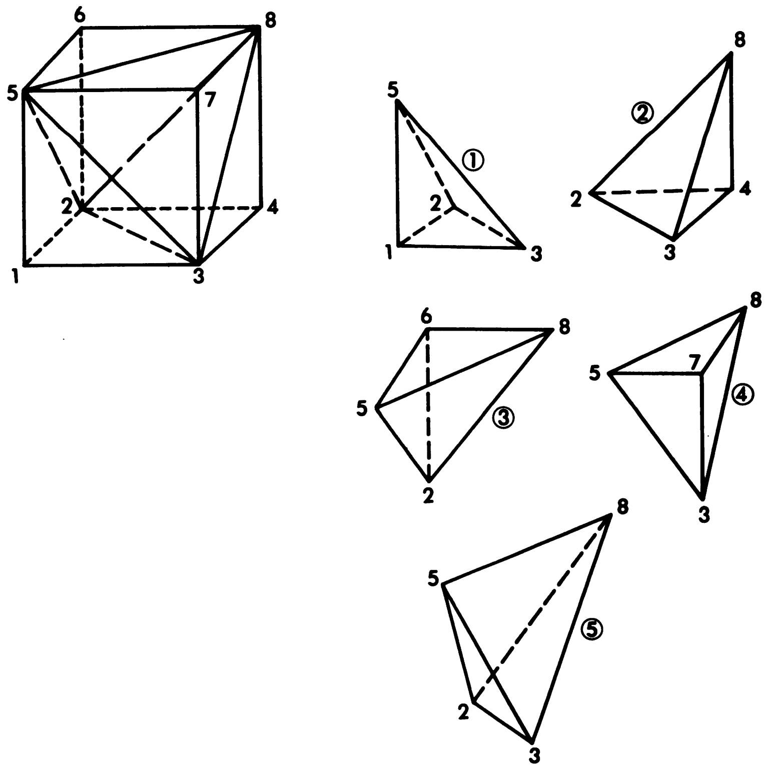
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Figure 7. Cubic domain subdivided into five tetrahedral finite elements, with eight nodes.

$$\begin{bmatrix} M_m \end{bmatrix} = -k_m^2 \begin{pmatrix} \int dV & \int x dV & \int y dV & \int z dV \\ \int x dV & (\int x^2 dV - \frac{1}{k^2}\int dV) & \int xy dV & \int xz dV \\ \int y dV & \int xy dV & (\int y^2 dV - \frac{1}{K^2}\int dV) & \int yz dV \\ \int z dV & \int xz dV & \int yz dV & (\int z^2 dV - \frac{1}{k^2}\int dV) \end{pmatrix} \tag{53}$$

The superscript T indicates the transpose matrix.

For further brevity, the right side of equation 50 is written as

$$\sum_m \frac{1}{\Delta_m^2} \left\{\bar{\phi}\right\}^T \begin{bmatrix} A_m \end{bmatrix} \left\{\bar{\phi}\right\} \tag{54}$$

In the example of the cube [Am] will be an 8 x 8 matrix and [ϕ] is an 8 x 1 column matrix (corresponding to the eight nodes). Each tetrahedron makes 16 contributions to the A-matrix. This is shown in figure 8 where two of the five tetrahedra are used for illustration to show where in [A] these contributions lie. The tetrahedra - filled cube makes 80 contributions to [A] as indicated at the bottom of figure 8. The surface integral in equation 14 requires the trial solution form

$$\hat{\phi}^{(s)} = \frac{1}{\Delta_s} (1 \; x \; y) \begin{bmatrix} G_s \end{bmatrix} \begin{pmatrix} \bar{\Phi}_1 \\ \bar{\Phi}_2 \\ \bar{\Phi}_3 \end{pmatrix} \tag{55}$$

since we are now dealing with triangular finite elements. (Equation 55 is analogous to equation 49).

Assuming a linearly polarized plane electromagnetic wave incident on one of the faces of the cube and using equation 15 and equation 16, the surface integral of equation 14 yields the form

$$\int (\alpha\phi + \beta\phi^2) dS = \sum_s \frac{ik_s}{\Delta_s^2} \left\{\bar{\Phi}\right\}^T \begin{bmatrix} G_s \end{bmatrix}^T \begin{bmatrix} M_s \end{bmatrix} \begin{bmatrix} G_s \end{bmatrix} \left\{\bar{\Phi}\right\} + \sum_{irrad} \frac{4ik_s}{\Delta_s^2} \left\{N_s\right\} \begin{bmatrix} G_s \end{bmatrix} \left\{\bar{\Phi}\right\} \tag{56}$$

where the ϕ, [G_S], and [M_S] are analogous with those of equation 50. The term containing [N_S], a 1 x 3 matrix, is caused by one of the faces being irradiated. Equation 56 is then combined with equation 54 into an expression with one main matrix, which now represents the functional (equation 14).

An exploded view of the six cube faces is shown in figure 9. It is seen that each face is subdivided into two triangles, and each triangle makes nine

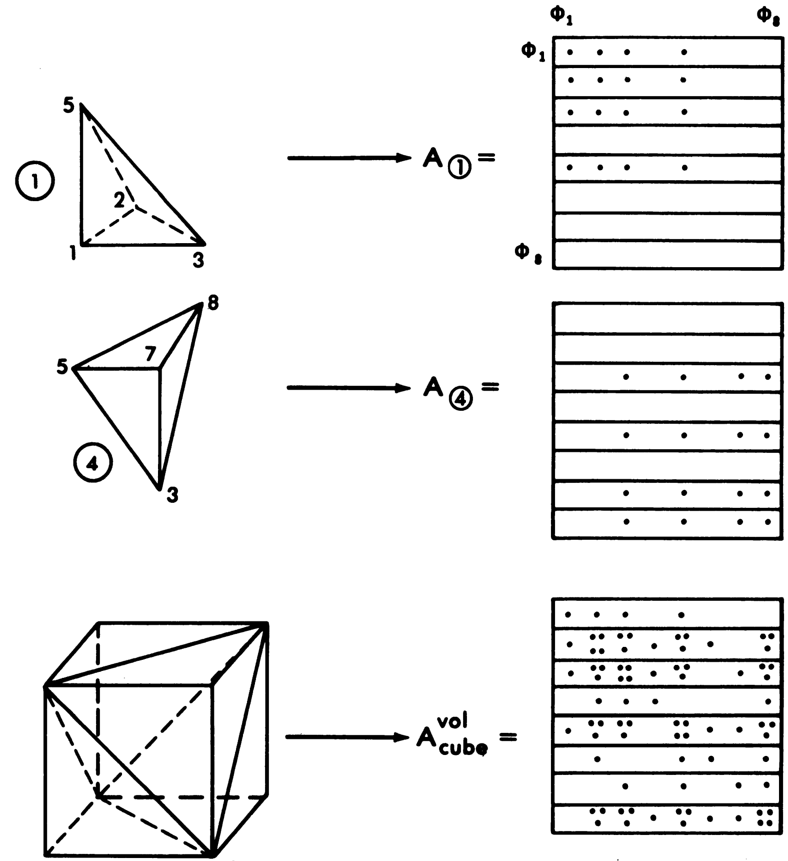
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Figure 8. Contribution to the (A) matrix from tetrahedral finite elements.

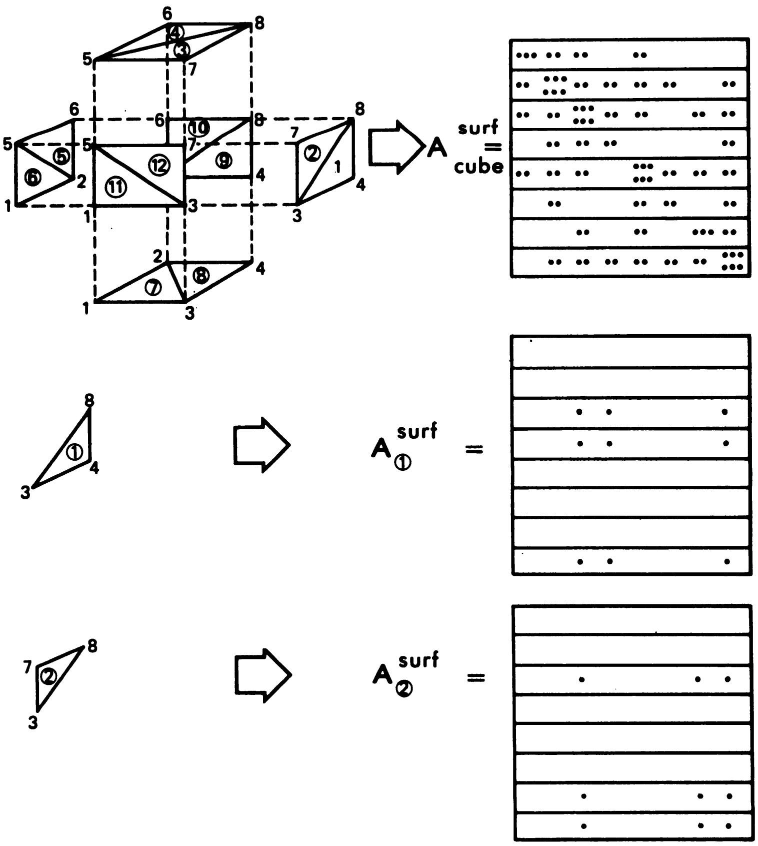
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Figure 9. Contribution to the (A) matrix from triangular finite elements.

contributions to the main matrix. (Only two triangles are shown for illustration purposes.) The irradiated face contributes terms linear in Φ as seen from equation 56. Thus, for the volume and surface of the cube the functional of equation 14 is replaced by the form

$$I = \sum_{i=1}^{8} \sum_{j=1}^{8} \bar{\phi}_i \; B_{ij} \; \bar{\phi}_j + \sum_{r=1}^{8} C_r \bar{\phi}_r \tag{57}$$

Equation 57 represents the combination of equations 54 and 56. The condition for minimizing this functional is

$$\frac{\partial I}{\partial \phi_i} = \sum_j A_{ij} \bar{\phi}_j + C_i = 0, \qquad i = 1, 2, \ldots, 8 \tag{58}$$

From equation 58 one obtains a system of linear algebraic equations in the unknown Φ_j, which is readily solved by the computer. This computation yields the values of the electric fields at the vertices of the cube.

An actual application is shown in figures 10 thru 12. A human thigh is modeled by a triple-layered cylinder with irregularly cross-section and infinite length. The thigh is irradiated by a TM wave (electric vector parallel to the long axis of the cylinder) of frequency 433 MHz, as shown in figure 10. The three regions are characterized by the dielectric properties of bone, muscle, and fat, respectively. The cross-section is subdivided into triangular finite elements. Each element and vertex is numbered for computer identification. Figure 11 shows this subdivision into 198 elements and 110 nodes. A plot of the magnitude of the electric field vector computed at each node is shown in figure 12. The calculated nodal values of $|E|$ are connected by linear interpolation.

CONCLUSION

A mathematical technique has been developed for calculating induced field distributions within irregularly shaped biological systems with complex internal structure. This technique makes use of the variational calculus, the method of trial solutions and the finite element method. A number of examples have been provided to acquaint the reader with these approximation techniques. A calculation of the induced internal fields has also been made on a triple-layered, irregularly shaped model of the human thigh exposed to 433 MHz plane-wave radiation. The results show high values of $|E|$ on the irradiated face with a rapid decrease as one progresses in the direction of propagation.

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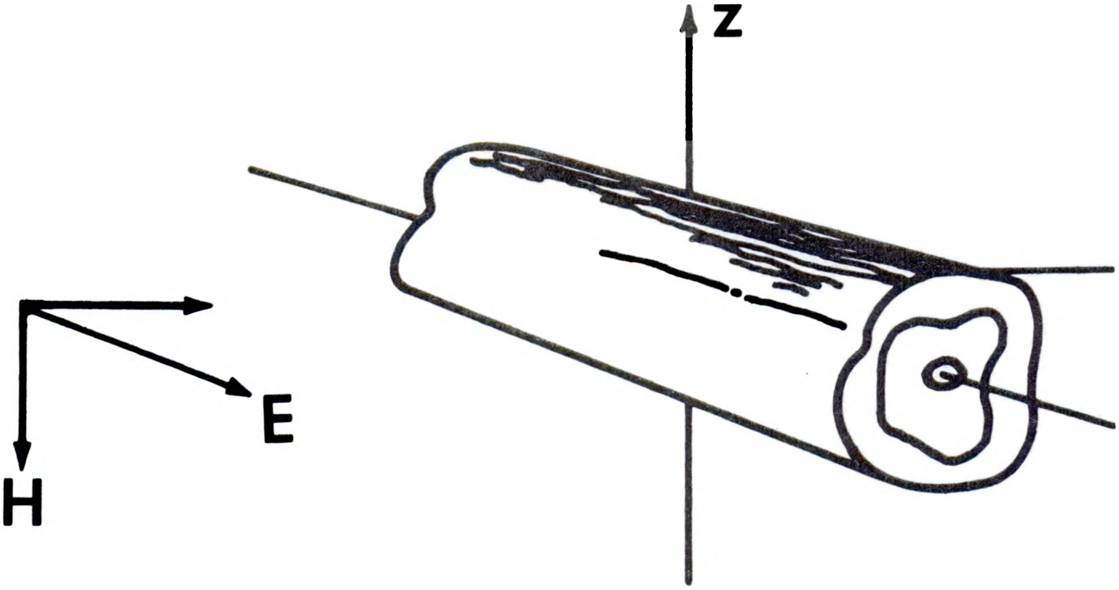

Figure 10. Triple-layered thigh model with irregular cross section, irradiated by TM 433 MHz field.

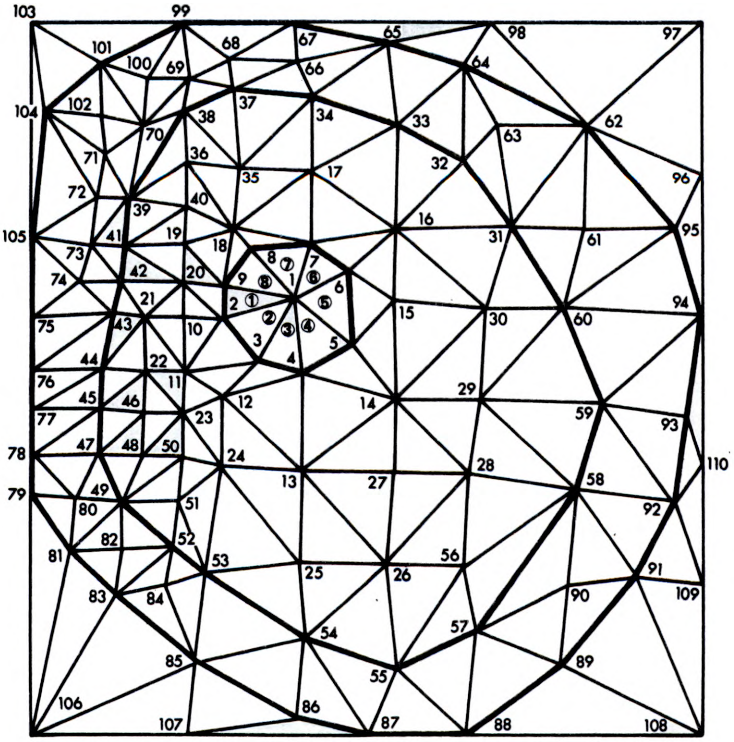
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Figure 11. Subdivision of a triple-layered, 17 cm x 20 cm thigh into 198 triangular elements and 110 nodes. (Eight of the 198 elements are identified by circled numbers). The rectangular enclosure is a mathematical region, assigned the properties of air.

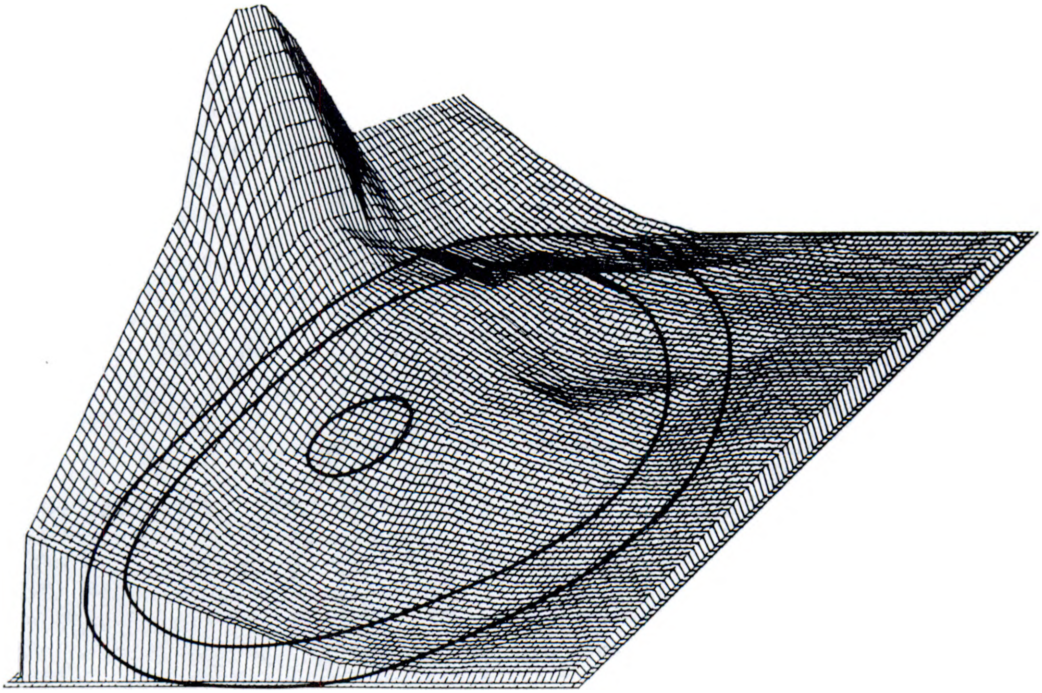

Figure 12. Electric field distribution within the thigh. (The three regions in the lower half of the figure correspond to fat, muscle, and central bone.)

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OPEN DISCUSSION

MR. WAXLER: Several years ago a friend of mine, Brian Robinson, made some impedance measurements--maps of the brain of a Rhesus monkey. What would be the implications of such impedance maps for this model? What would one do with such impedance maps? Could it tell you where hot spots would be?

MR. WAXLER: If one could relate that to the dielectric properties, one could compare a theoretical calculation with that mapping; that would be one way to verify the mathematical model as well as the experimental results. However, hot spots depend upon not only the dielectric properties but upon the geometry, as well. In that sense, just from knowing dielectric properties, you could not predict hot spots. There is more coming into play besides the dielectric properties: one has to consider the geometry, both shape and neighboring structures, because of the focusing and reflection effects and resonances or interference possibilities.

MR. TAYLOR: What is the physical significance of the function. In at least the static case, it looked like the energy.

MR. NEUDER: Different problems have different functionals, and one could ascribe that the physical significance be, for example, energy. Do you want to minimize energy? Problems of stable equilibrium are governed by the variational principle of the minimum potential energy.

MR. TAYLOR: Yes, that's what it looked like in the static case.

COMPARISON OF CALCULATED ABSORBED DOSE RATE DISTRIBUTIONS IN PHANTOM HEADS EXPOSED TO 2450 MHz AND 915 MHz PLANE WAVE AND SLOT SOURCES

Henry S. Ho
Division of Biological Effects
Bureau of Radiological Health, FDA

ABSTRACT

Calculations are made for the plane wave and slot source induced absorbed dose rate patterns in 7 cm and 3.3 cm phantom heads simulated by dielectric spheres. The deeper penetration due to the plane wave source (whole body) exposure, contrasting the tenfold larger maximum absorbed dose rate due to slot source (partial body) exposure, illustrates the vast difference in energy absorption patterns due to these two types of sources for both the 915 MHz and 2450 MHz source frequencies. An increase in penetration depth with increased slot width is noted for both source frequencies, while a change in source frequency from 2450 MHz to 915 MHz does not greatly change the penetration depth for a given slot size.

INTRODUCTION

Current microwave absorbed dose rate studies concentrate on radiation fields simulated by plane waves which expose the whole body of biological subjects. In terms of possible health hazard considerations, however, exposures usually are considered more hazardous at locations close to the radiation source. One reason is that the power density decreases proportionately to the square of the distance from the source. At locations near a microwave radiation source, the exposure field often approximates that of an aperture or slot source. This type of exposure field often results in partial-body exposure in contrast to the whole-body exposure due to plane wave sources. Examples of this type of exposure field include the leakage field near a microwave oven door and the field near a microwave diathermy applicator. It has been reported that the absorbed dose rate patterns induced by an aperture source differs significantly from that of plane wave sources at 2450 MHz (1). In the present investigation, absorbed dose rate patterns in phantom head due to 915 MHz and 2450 MHz plane wave and slot sources are calculated. Several widths of the slot sources narrower than that used in the previous investigation are chosen to simulate more closely the field near a leaking microwave oven door.

METHODS

The methods for calculating the induced absorbed dose rate (mW/g) in an aperture irradiated dielectric sphere simulating human and monkey heads has been reported (1). Calculations of absorbed dose rate in plane wave irradiated dielectric spheres were also reported (2). The positions of the slot and plane wave sources with respect to a phantom head are shown in figure 1.

In the present investigation, calculations are made for two single-layered brain spheres of radii 7 cm and 3.3 cm, and a five-layered phantom head of outer radius equal to 3.3 cm. Four sizes of slot sources are used. The lengths of

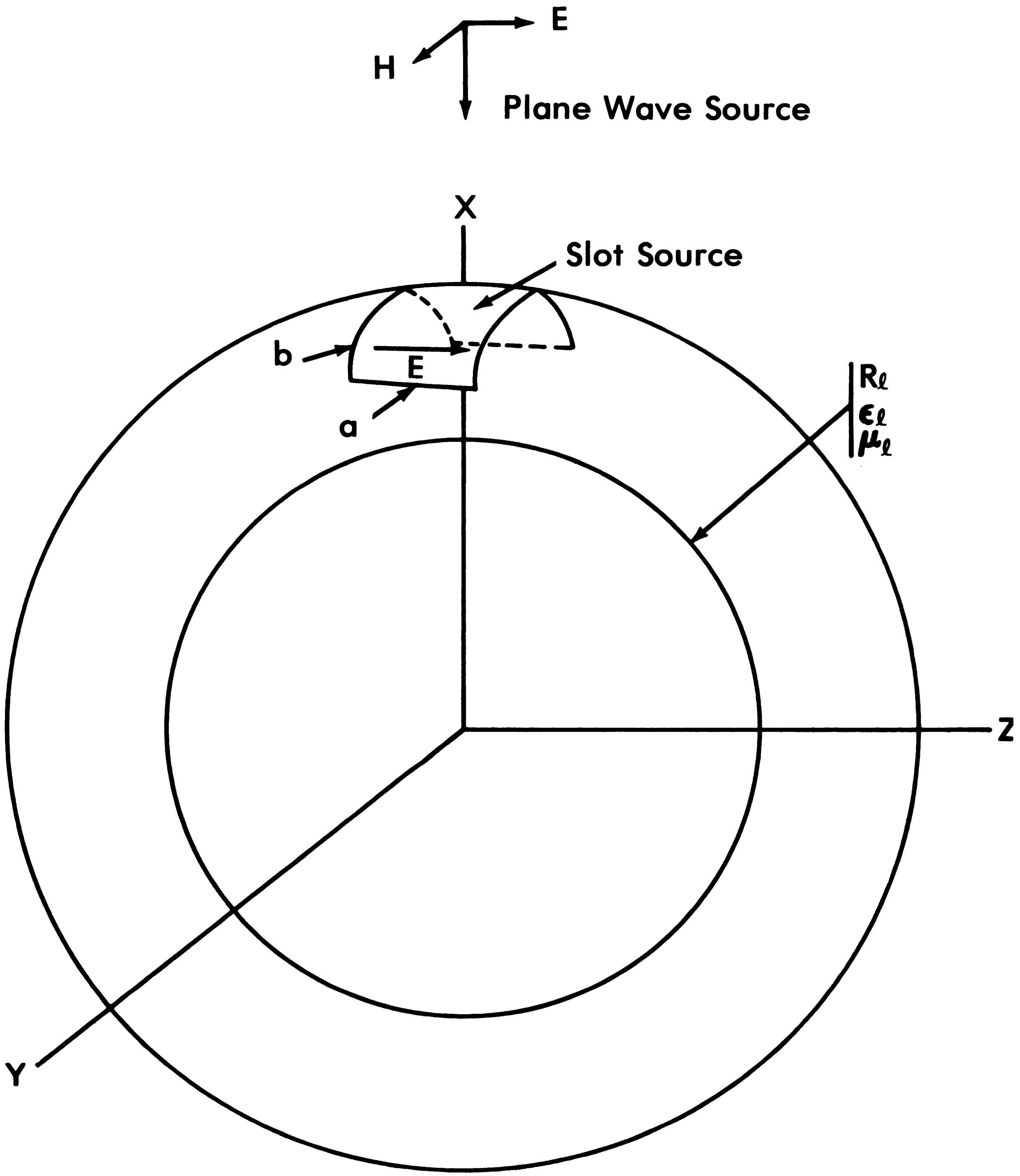
E
H
Plane Wave Source
X
Slot Source
b
E
a
R_ℓ
ϵ_ℓ
μ_ℓ
Z
Y

Figure 1. Multilayered dielectric sphere with directions and positions of incident plane wave and slot source.

the slots are constant at 7 cm while the widths are 4.5, 2.0, 1.0 and 0.5 cm respectively. Source frequencies used are 2450 MHz and 915 MHz. The frequency-dependent dielectric constants of the phantom heads used for the present calculation are the same as those reported in previous investigation (1,4,5).

RESULTS

Figures 2(a) and 2(b) show the absorbed dose rate pattern on the X-Z and X-Y planes of the 7 cm radius phantom head due to a 7 by 4.5 cm aperture source with a microwave frequency of 915 MHz. Figures 3(a) and (b) show the absorbed dose rate patterns due to the same aperture source in the 3.3 cm radius single-layered phantom head. These results indicate a wedge type of penetration of microwave energy by the 915 MHz aperture source similar to that caused by the previously reported 2450 MHz aperture source (1). Even though the absolute microwave penetration depths for the 3.3 cm and 7 cm phantom head are approximately the same, it is important to note that the penetration, in terms of relative position in the phantom head, is deeper for the smaller phantom head. Figures 4(a) and (b) indicate the absorbed dose rate patterns in the 7 cm radius phantom head exposed to a 915 MHz plane wave source. The much deeper penetration and high concentration of absorption in small regions interior to the phantom head contrast with the wedge type of penetration due to the aperture source. Another important difference between the absorptions due to these two different sources, however, is the maximum absorbed dose rate. The energy rate absorption for the aperture source has a maximum of 3.77 mW/g compared to the 0.41 mW/g for the plane wave case, given the same exposure electric field squared of 3770 V^2/m^2. This value of electric field squared corresponds to a free space power density of 1.0 mW/cm^2. These observations are similar to those reported for the 2450 MHz case (1). With a decrease in the width of the aperture source so that it approaches a narrow slot source, the penetration of energy absorption decreases as shown in figure 5. A similar dependence of penetration of energy absorption on slot size is also found for the 2450 MHz case. Tables 1, 2, and 3 summarize the maximum and average absorbed dose rates in the phantom heads due to plane wave and slot sources.

Table 1. Maximum and average absorbed dose rate in 7 cm radius phantom head due to plane wave and slot sources

	2450 MHz		915 MHz	
Radiation Source	Dmax (mW/g)	Davg (μW/g)	Dmax (mW/g)	Davg (μW/g)
Plane wave	0.420	93.10	0.414	117.0
Slot 4.5 x 7 cm	5.160	68.60	3.770	71.80
Slot 2 x 7 cm	6.070	29.40	3.880	27.60
Slot 1 x 7 cm	2.710	12.20	1.730	8.840
Slot 0.5 x 7 cm	0.788	3.600	0.505	2.380

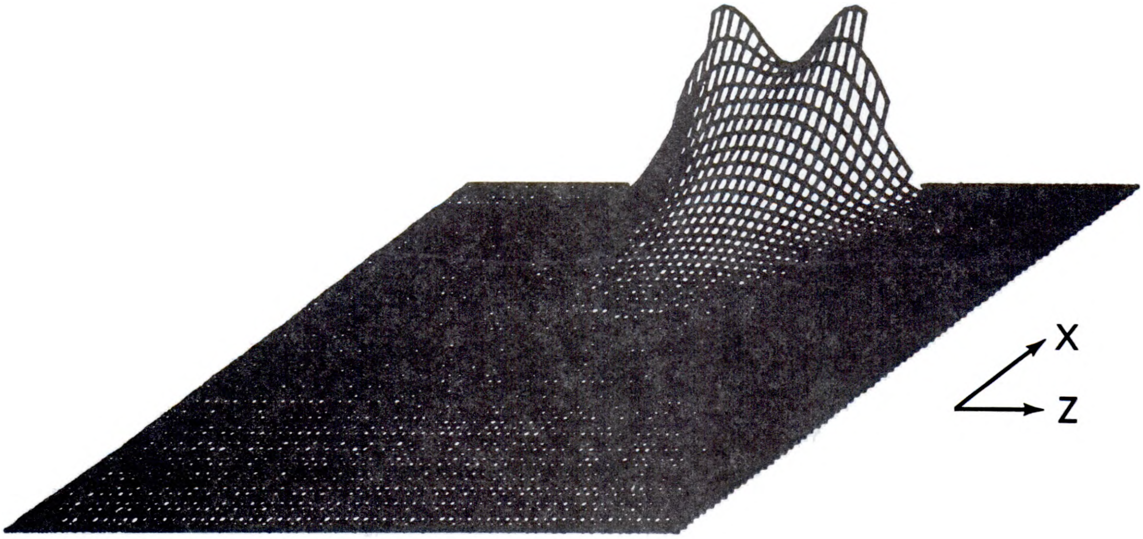

Figure 2a

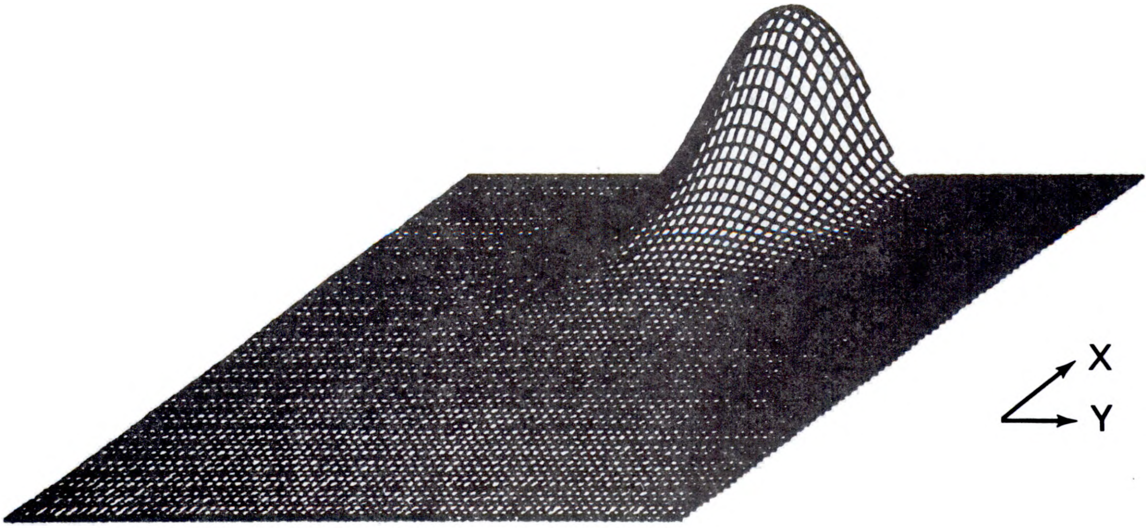

Figure 2b

Figures 2a and 2b. The absorbed dose rate patterns of the 7 cm radius phantom head due to a 915 MHz 7 by 4.5 cm aperture source, in the (a) X-Z plane, and (b) X-Y plane. Maximum absorbed dose rate = 3.77 mW/g. Exposure E^2 = 3770 V^2/m^2.

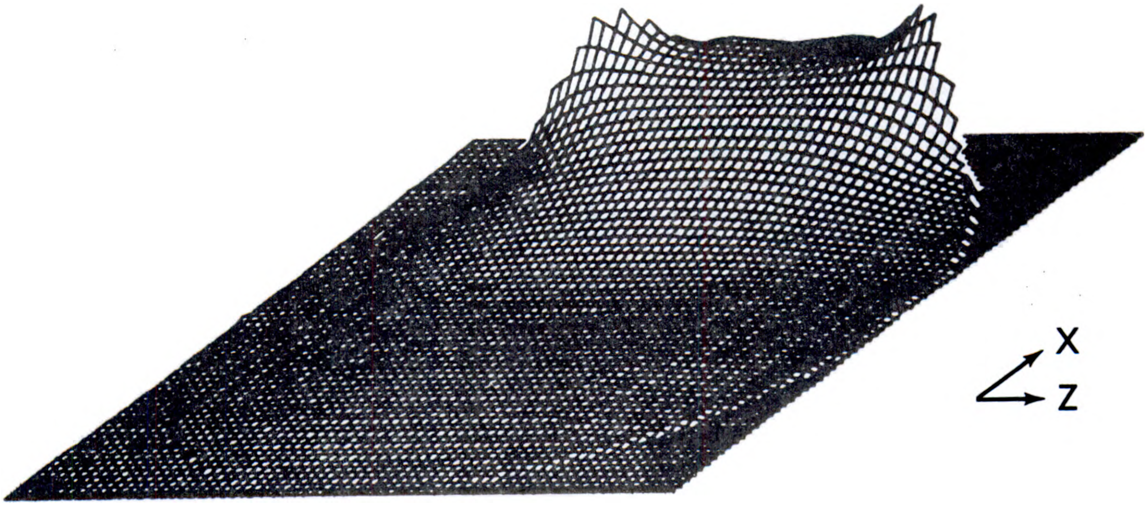

Figure 3a

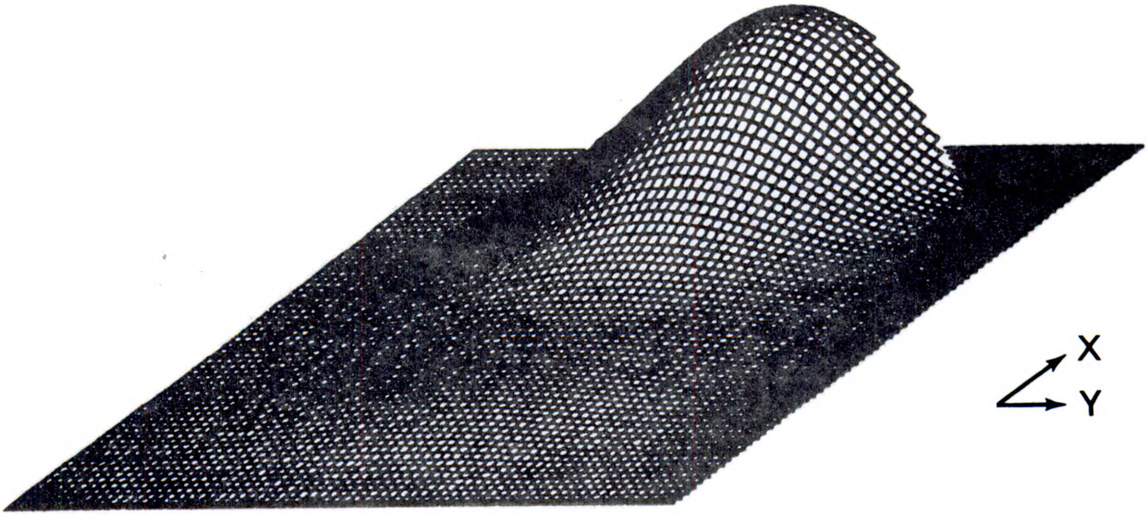

Figure 3b

Figures 3a and 3b. The absorbed dose rate patterns of the 3.3 cm radius phantom head due to a 915 MHz 7 by 4.5 cm aperture source, in the (a) X-Z plane, and (b) X-Y plane. Mzximum absorbed dose rate = 4.53 mW/g. Exposure E^2 = 3770 V^2/m^2.

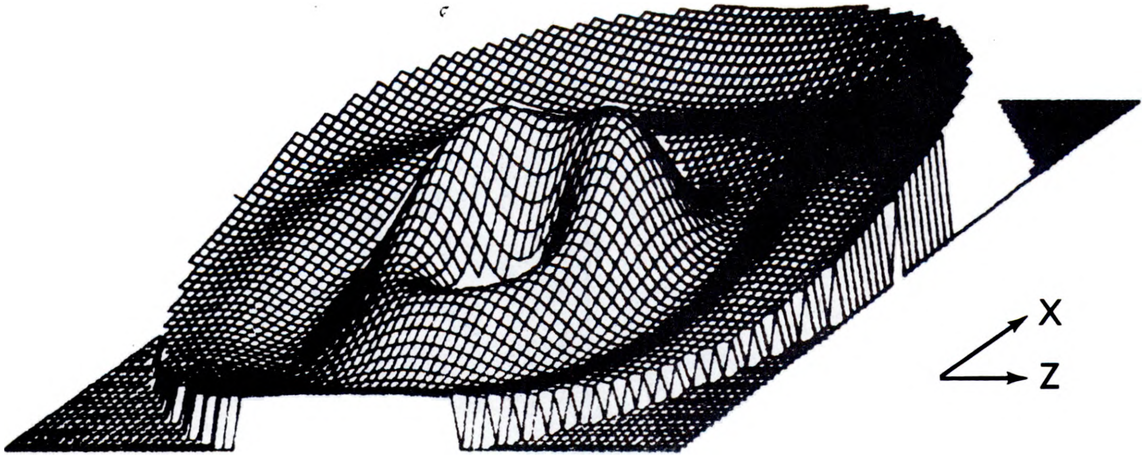

Figure 4a

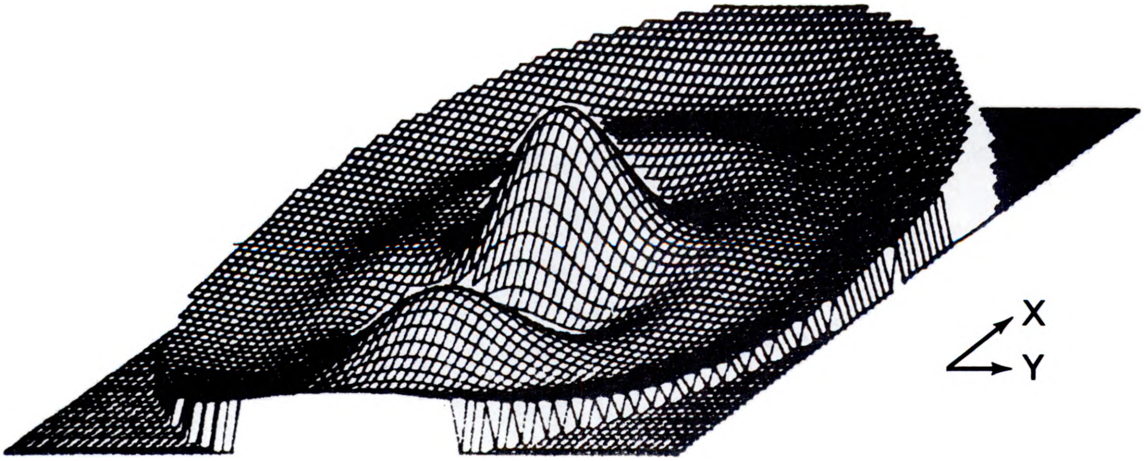

Figure 4b

Figures 4a and 4b. The absorbed dose rate patterns of the 7 cm radius phantom head due to a 915 MHz plane wave source, in the (a) X-Z plane, and (b) X-Y plane. Maximum absorbed dose rate = 0.414 mW/g. Exposure E^2 = 3770 V^2/m^2.

Table 2. Maximum and average absorbed dose rate in 3.3 cm radius phantom head due to plane wave and slot sources

Radiation Source	2450 MHz Dmax (mW/g)	2450 MHz Davg (μW/g)	915 MHz Dmax (mW/g)	915 MHz Davg (μW/g)
Plane wave	1.840	248.0	0.909	261.0
Slot 4.5 x 7 cm	5.470	547.8	4.530	552.0
Slot 2 x 7 cm	4.814	261.4	2.695	225.1
Slot 1 x 7 cm	6.321	123.7	4.038	75.81
Slot 0.5 x 7 cm	3.056	41.09	1.953	22.06

Table 3. Maximum and average absorbed dose rate in 3.3 cm radius, 5-layered phantom heads due to plane wave and slot sources

Radiation Source	2450 MHz Dmax (mW/g)	2450 MHz Davg (μW/g)
Plane wave	1.480	249.0
Slot 4.5 x 7 cm	10.44	430.0
Slot 2 x 7 cm	8.536	214.0
Slot 1 x 7 cm	13.15	81.36
Slot 0.5 x 7 cm	6.231	25.22

In order to compare the relative energy absorption penetration in the phantom heads due to the different sizes and frequencies of the slot sources, a one number index called penetration depth (pd) is used. This index (pd) is computed by determining the radial distance from the surface toward the center of the phantom head to a point beneath which the absorbed dose rate is less than an assigned value (Dd). Figures 6, 7, and 8 show the penetration depth (pd) for the three phantom heads as a function of slot width for assigned absorbed dose rate (Dd) values of 1.0 and 0.1 mW/g; and source frequencies of 2450 MHz and 915 MHz. The results indicate increased penetration depth (pd) with increased slot width. A smaller penetration depth results from the same size 915 MHz slot

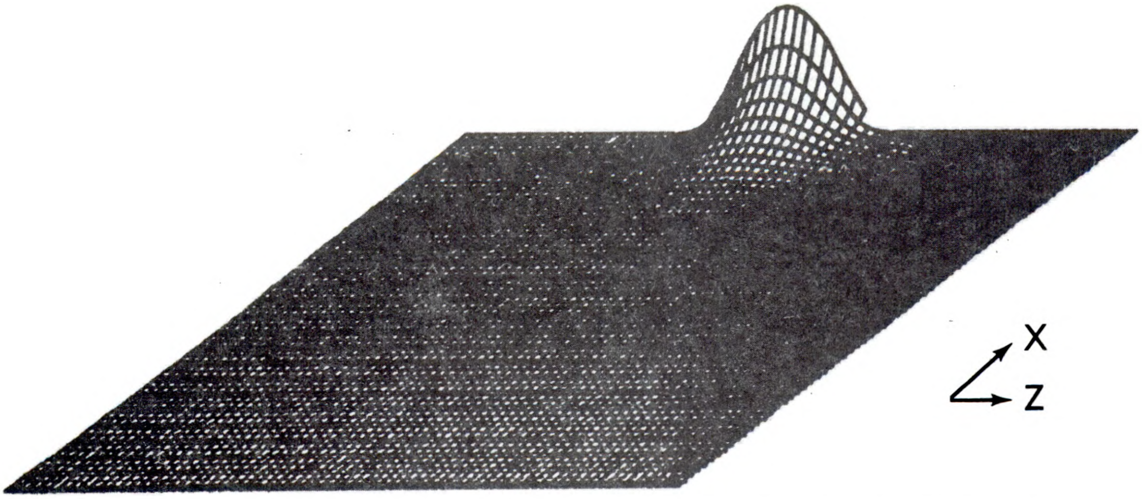

Figure 5. The absorbed dose rate pattern of the 7 cm radius phantom head due to a 915 MHz 7 by 1.0 cm slot source on the X-Z plane. Maximum absorbed dose rate = 1.73 mW/g. Exposure E^2 = 3770 V^2/m^2.

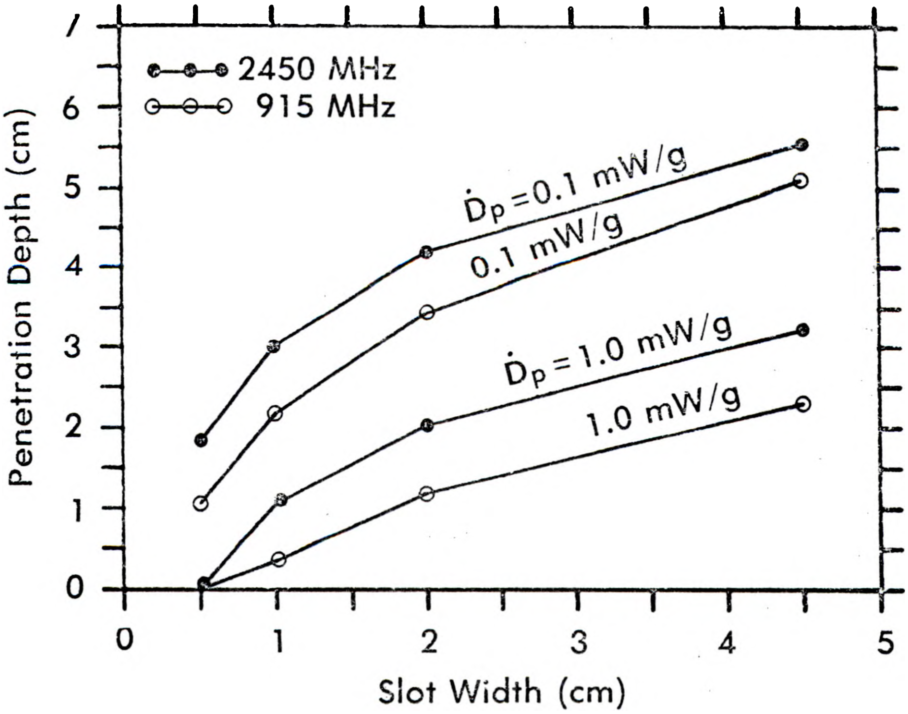

Figure 6. The penetration depths (pd) for the 7 cm radius phantom head versus aperture width for assigned absorbed dose rates (Dp) of 1.0 and 0.1 mW/g; and source frequencies of 915 MHz and 2450 MHz. Exposure E^2 = 3770 V^2/m^2.

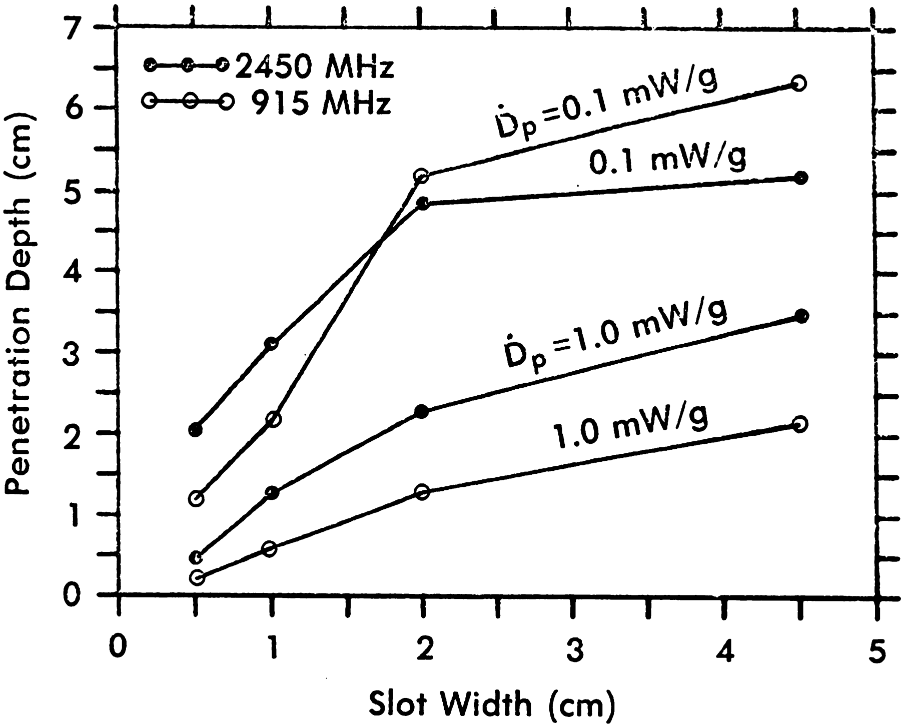

Figure 7. The penetration depth (pd) for the 3.3 cm single-layered phantom head versus aperture width for assigned absorbed dose rates (Dp) of 1.0 and 0.1 mW/g; and source frequencies of 915 MHz and 2450 MHz. Exposure E^2 = 3770 V^2/m^2.

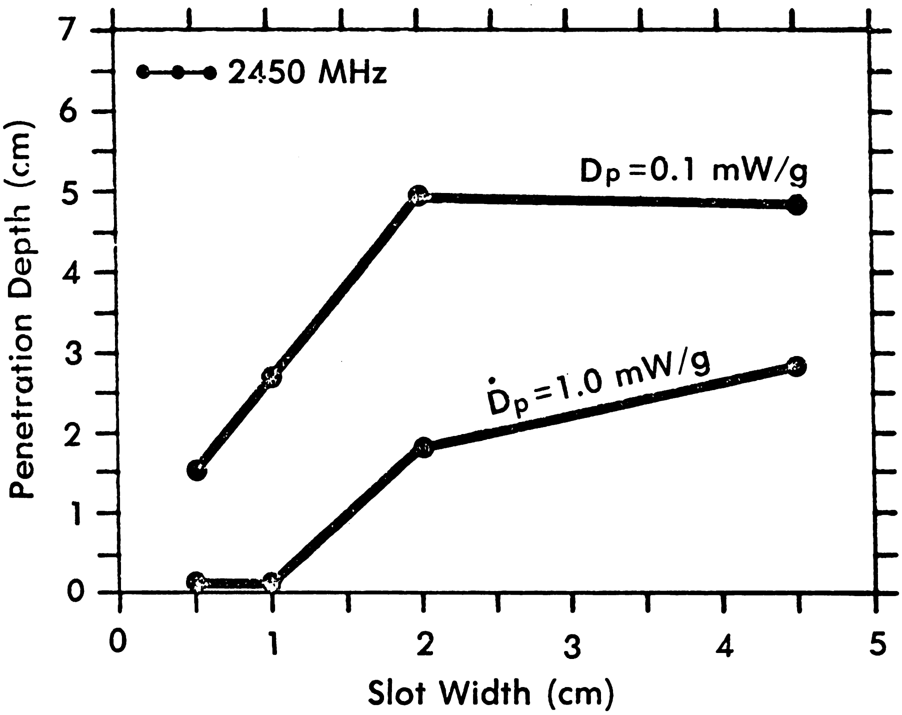

Figure 8. The penetration depth (pd) for the 3.3 cm five-layered phantom head versus aperture width for assigned absorbed dose rates (Dp) of 1.0 and 0.1 mW/g; and source frequency of 2450 MHz. Exposure E^2 = 3770 V^2/m^2.

compared to that of the 2450 MHz aperture. This apparent contradiction with expected results from the skin depth consideration is explained by the fact that slots with the same physical sizes are wavelength-wise smaller for the 915 MHz frequency than the 2450 MHz. Hence for a given physical size of aperture source, a decrease in source frequency from 2450 MHz to 915 MHz results in an increase in 'skin depth' penetration as well as a decrease in effective slot size. ('Skin depth' is a standard engineering quantity which is dependent on source frequency and dielectric properties of the media.) These two factors counterbalance each other resulting in a small net change in the penetration depth (pd).

DISCUSSION

The results of this theoretical investigation indicate completely different patterns of microwave energy absorption between the plane wave (whole body), and the slot source (partial body) exposure. Plane wave exposure results in a deeper penetration while the slot source exposure results in a larger maximum absorbed dose rate.

The penetration depth (pd) due to the slot source increases with increased slot width. For a given aperture size, the change in penetration depth (pd) is small when the source frequency decreases from 2450 MHz to 915 MHz because of the counterbalancing effects of increased 'skin depth' and decreased 'effective' slot size.

An important question remains as to how close the results of this investigation simulates that of the real life exposure of human heads to microwave slot sources. One parameter of concern would be, for example, the 'effective' slot size of the exposure field from a leaking microwave oven door as a function of distance from the door. The changes in absorption due to the irregular geometry of the human head are another concern. A physical measurement of absorption using real human skulls, phantom tissues, actual microwave ovens and the newly developed implantable miniature electric field probe (3) perhaps will add to the results of this investigation towards the health hazard evaluation of leaking microwave slot sources.

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CHANGE IN AVERAGE ABSORBED DOSE RATE OF A GROUP OF MICE UNDER REPEATED EXPOSURE TO 915 MHz MICROWAVE RADIATION

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ABSTRACT

Reduction of microwave energy absorption by individual animals during single exposure to 2450 MHz CW radiation in the waveguide has been reported recently. In the present investigation two groups of mice (14 males or 14 females) were irradiated 8 hours per day during alternate working days of the week for a total period of two months. The air temperature, relative humidity, and air flow rate in the waveguide were 20-28° C, 40-50 percent and 37 liters/min respectively. For a forward power of 5W which resulted in an average absorbed dose rate of approximately 5 mW/g, a reduction of energy absorption after the first hour of each session was observed throughout the experiment for both groups of animals. A lower average absorbed dose rate was also observed for the first several sessions of the irradiation, compared to those of the remaining sessions. The result indicates possible problems in dosimetry determination for animal irradiation apparatus where parameters for the determination of average absorbed dose rate are not monitored during the irradiation.

INTRODUCTION

It has been reported that mice singly irradiated in a waveguide with 2450 MHz CW microwave radiation will reduce their microwave energy absorption with average absorbed dose rate as little as 28 mW/g in an environment of 24° C temperature, 50 percent relative humidity and air flow rate of 38 liters/min (1). In another experiment, the reduction of microwave energy absorption was observed with average absorbed dose rate of 23.6 mW/g, and environmental conditions of 24° C temperature, 55 percent relative humidity, and an air flow rate of 78 ml/min (2). However, these reported experiments are for single exposure of individual animals over relatively short durations of 15 to 30 minutes. In the present investigation, two groups of 14 mice each were repeatedly irradiated, in 8-hour sessions, with 915 MHz CW microwave radiation to determine possible changes in microwave energy absorption by the mice during each irradiation session and through-out the experiment.

MATERIALS AND METHODS

The microwave exposure system is a 915 MHz WR 975 waveguide apparatus with a cross section of 24.8 by 12.4 cm. A block diagram of the irradiation apparatus is shown in figure 1. The power fed into (P_f), reflected from (P_r), and transmitted through (P_t) the animal are measured by power meters through the directional couplers. Electronic signals from the power meters are time-integrated by the three-channel voltage integrator. The average absorbed dose rate of the animal for a given time interval is shown in equation (1)

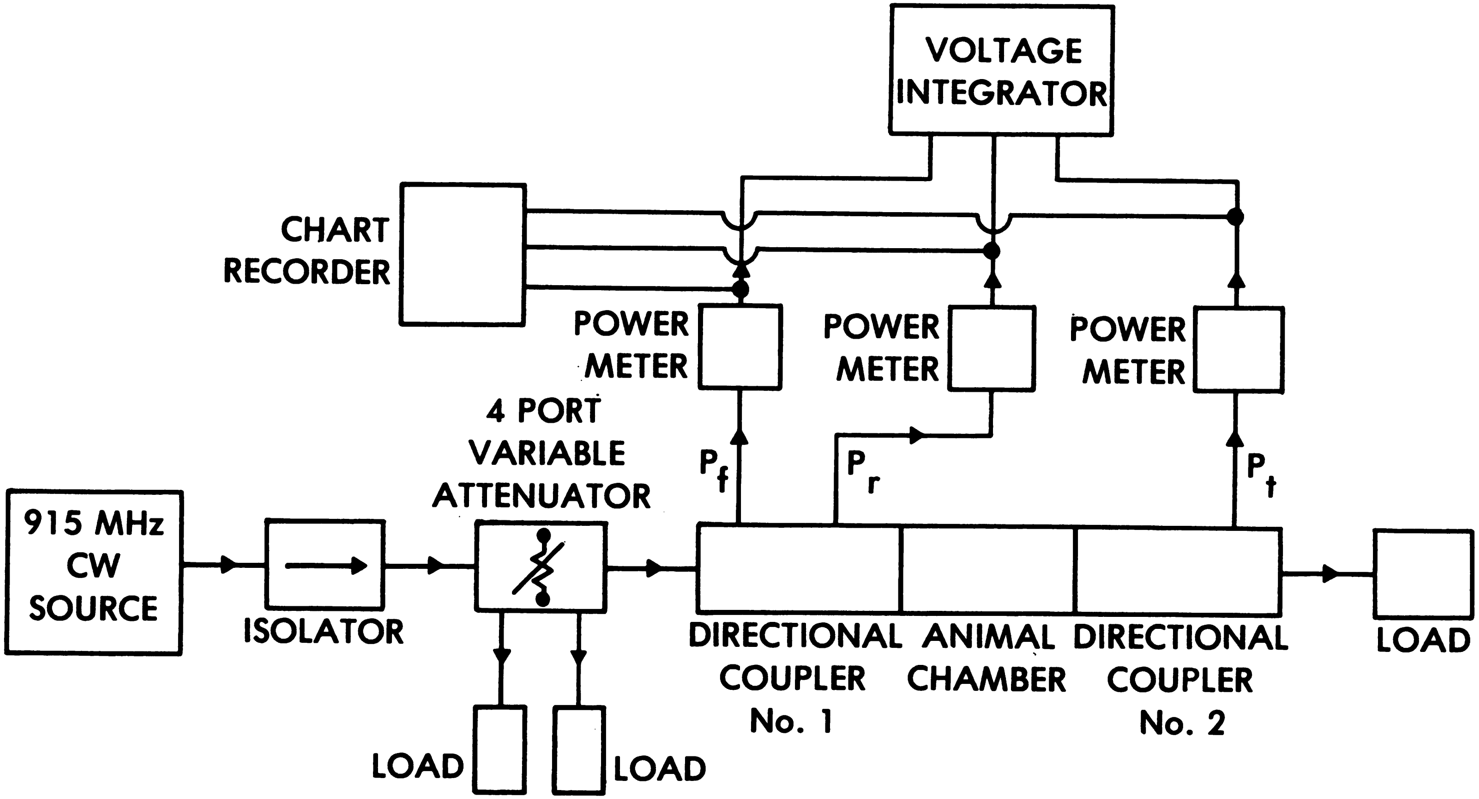
VOLTAGE INTEGRATOR
CHART RECORDER
POWER METER
POWER METER
POWER METER
4 PORT VARIABLE ATTENUATOR
P_f
P_r
P_t
915 MHz CW SOURCE
ISOLATOR
DIRECTIONAL COUPLER No. 1
ANIMAL CHAMBER
DIRECTIONAL COUPLER No. 2
LOAD
LOAD
LOAD

Figure 1. Block diagram of the waveguide irradiation apparatus.

$$D_{avg} = \frac{\int_0^T P_f\,dt - \int_0^T P_r\,dt - \int_0^T P_t\,dt}{(T)(Wt)} \quad \text{mW/g,} \qquad (1)$$

where D_{avg} is the average absorbed dose rate, T is the exposure time interval, and Wt is the total weight of the animals in grams.

Within the waveguide, the animals are housed in a holder 23 by 21 by 9 cm inner diameters made of Plexiglas 3.0 mm thick ($\epsilon_r = 2.6$, $\tan \delta = 0.00057$). Two opposite walls of the holder are made of a grid of plastic strips to allow air to flow through the holder. Air was blown in from an opening at one end of the waveguide and exhausted through the opposite end of the apparatus. An air flowmeter monitors the rate of the air flow. The temperature of the exhasuted air and the room humidity were also monitored.

Four-week old mice were divided into two groups of 14 male and 14 females. The two groups of animals were irradiated 8 hours per day during alternate working days of the week for a total period of two months. The male group had a total of 22 irradiation sessions, and the female group had 21. The air temperature, relative humidity, and air flow rate in the waveguide were 20-28° C, 40-50 percent and 37 liters/min respectively. The average absorbed dose rate for each group of animals was determined at one-hour intervals. The forward power was 5 W. Because the animals were growing, the total weight of each group of animals increased with time. Figures 2(a) and 2(b) show the total weight versus the irradiation session for the male and the female groups of animals. Figures 3(a) and 3(b) show the air temperature versus exposure duration, and irradiation session of the male and female groups irradiated with a forward power of 5 W. Figures 4(a) and 4(b) show the air temperature for each irradiation hour averaged over all irradiation sessions for each group of animals.

As a preliminary experiment, another group of 14 female mice with a total weight of 365 g were irradiated with a lower forward power of 2.5 W, 6 hours per day for 4 days.

RESULTS

The average absorbed dose rate versus exposure duration in each session, and irradiation session, of the male and female groups irradiated with a forward power of 5 W, and the female group irradiated with a forward power of 2.5 W is shown in figures 5(a), 5(b) and 6. The values for each of the irradiation hours are then averaged over irradiation sessions.

The mean and standard error of the mean (S.E.M.) of the average absorbed dose rate of the female group of mice irradiated with a forward power of 2.5 W are shown in figure 7. With a power of 2.5 W which resulted in a mean average absorbed dose rate of 3.4 mW/g, there is no reduction in microwave energy absorbtion during the irradiation. Figures 8(a) and 8(b) show the mean and S.E.M. of the male and female groups of mice irradiated with a forward power of 5 W. The microwave energy absorption decreased from a mean value of 6.9 mW/g during the first hour to a low of 4.3 mW/g during the sixth hours and then increases slightly to 4.9 mW/g during the eighth hour for the male mice. The energy absorption of the female group changed in a similar manner during microwave irradiation with mean values of 7.3, 4.6 and 4.9 mW/g during the first, sixth and eighth hour respectively. This change in energy absorption during the microwave irradiation cannot be accounted for by the much smaller change in air temperature shown in figures 4(a) and 4(b).

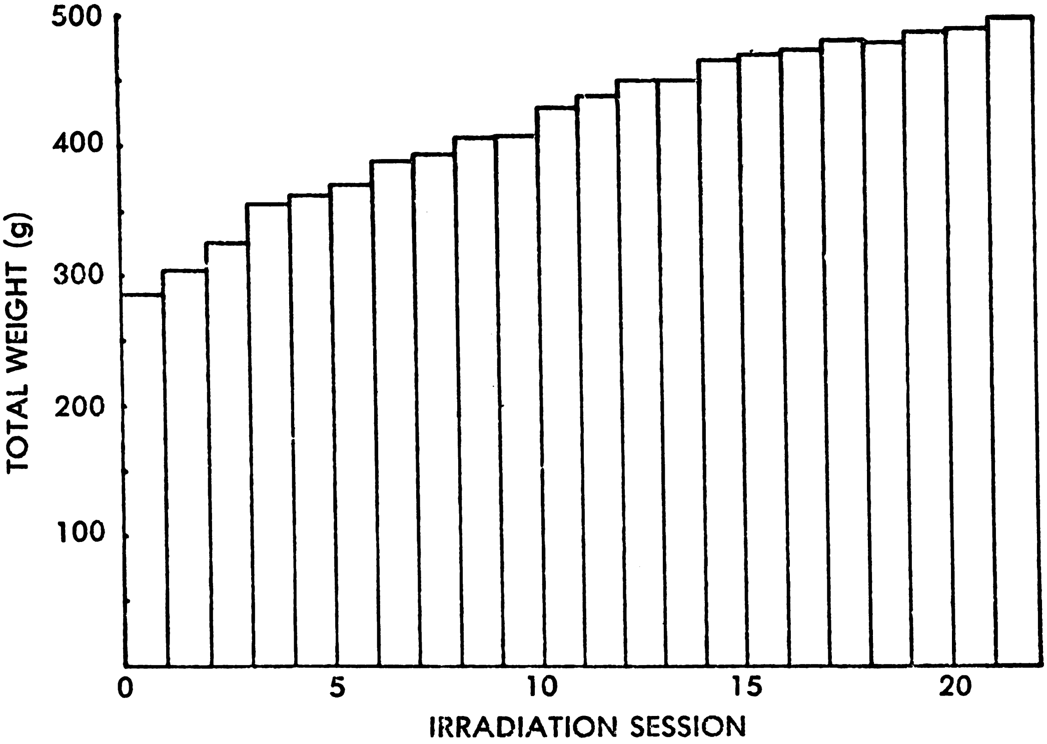

Figure 2a

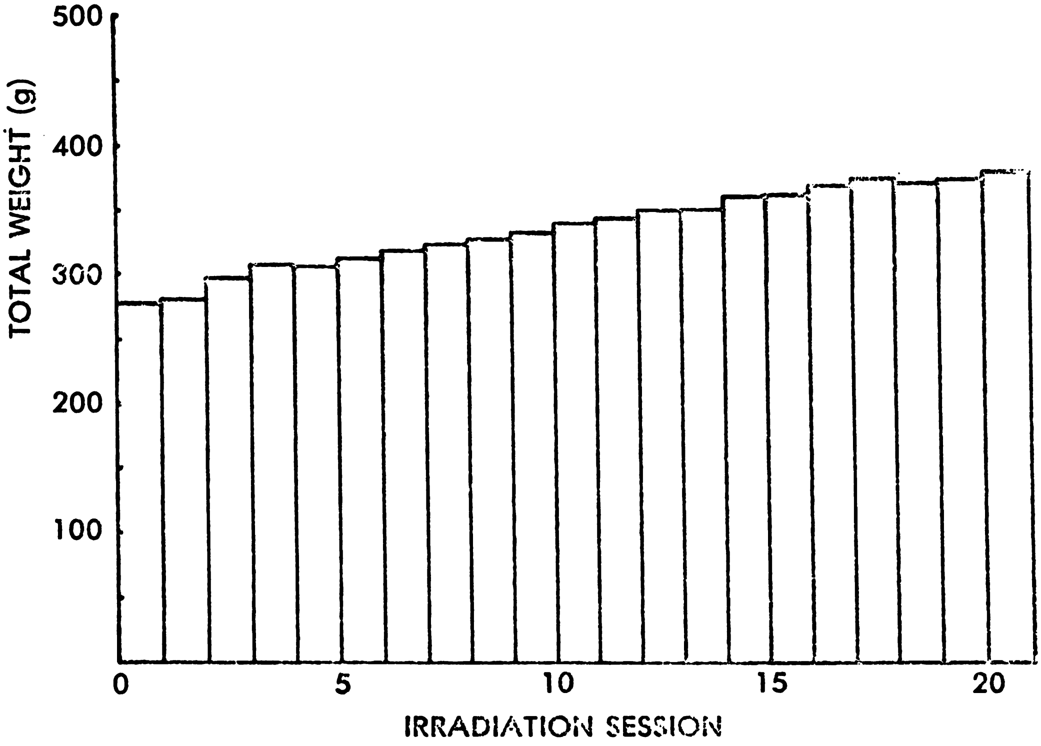

Figure 2b

Figures 2a and 2b. Total weight of the (a) 14 male mice, and (b) 14 female mice versus irradiation session.

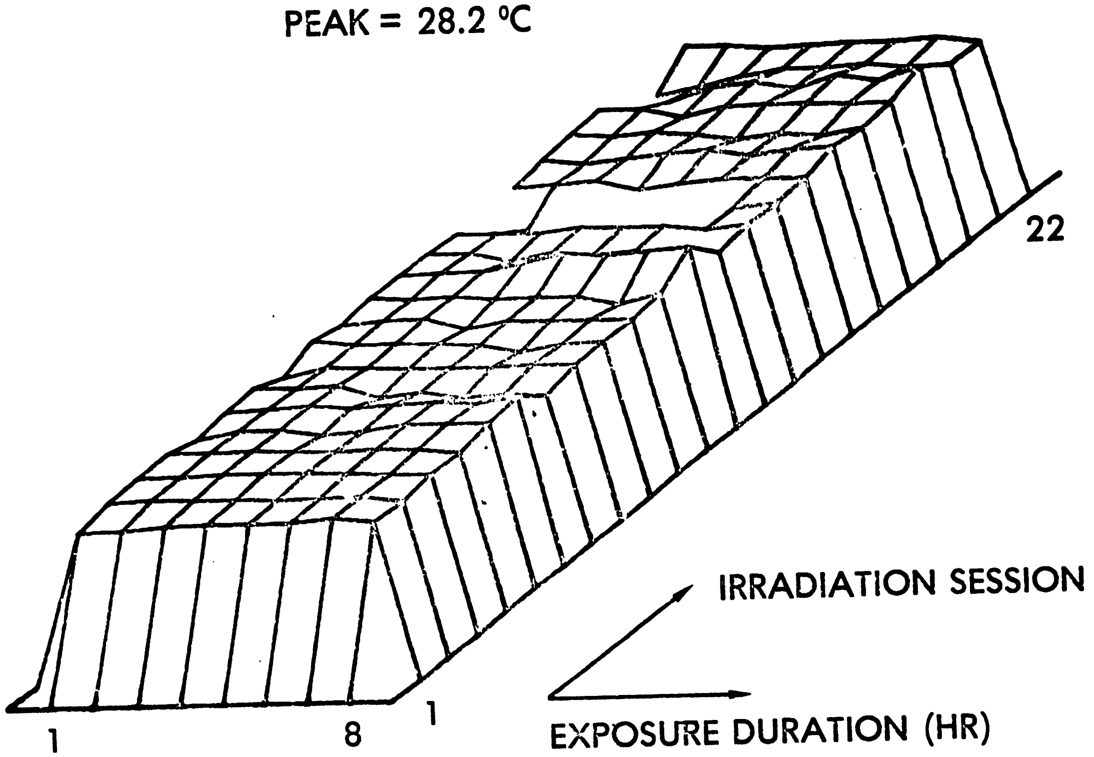

Figure 3a

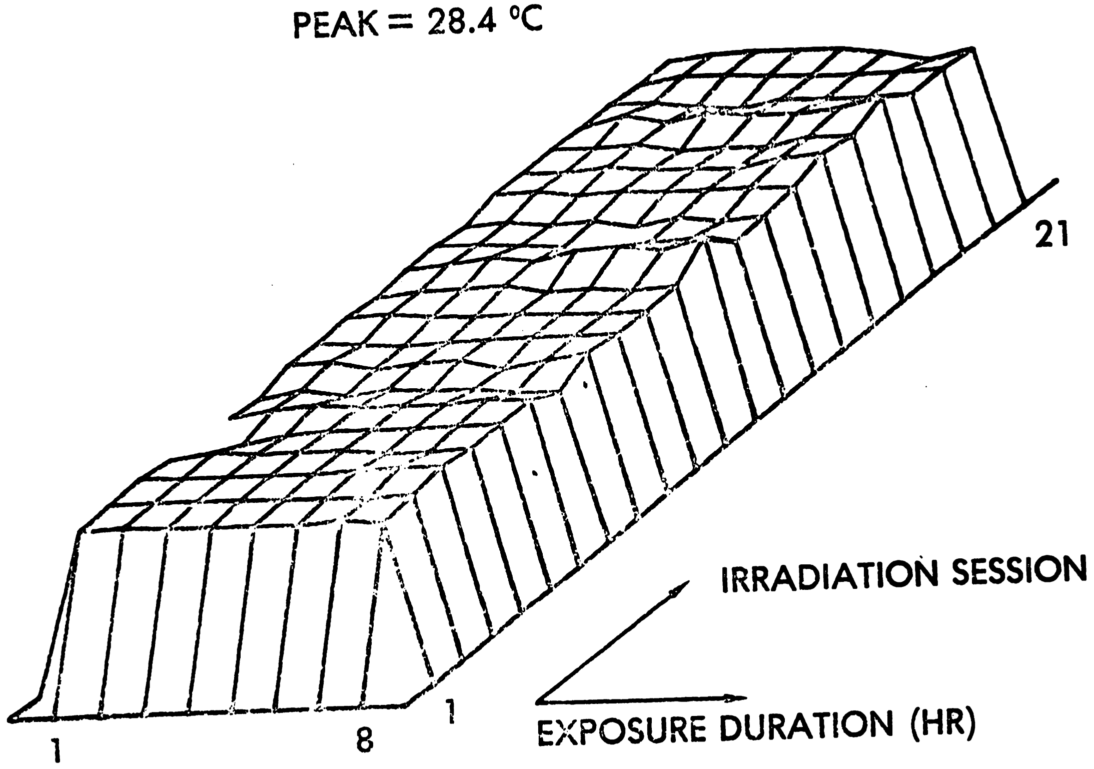

Figure 3b

Figures 3a and 3b. The air temperature versus exposure duration, and irradiation session of (a) 14 male mice, and (b) 14 female mice irradiation with a forward power of 5 W.

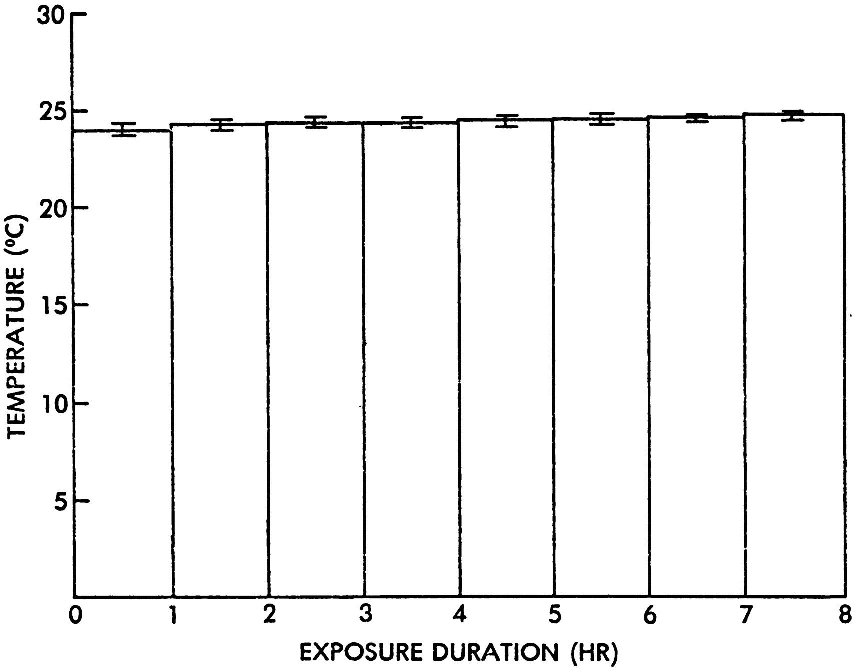

Figure 4a

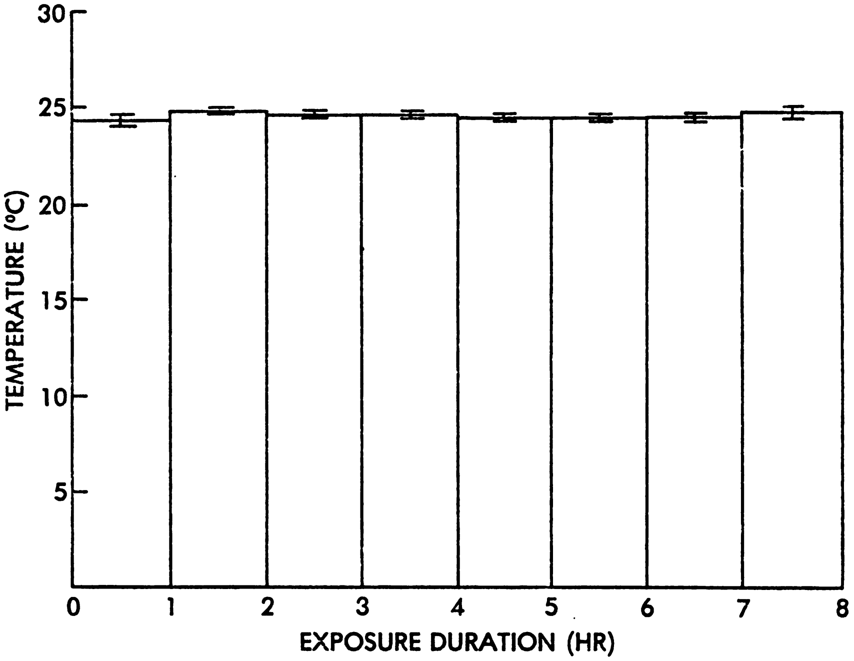

Figure 4b

Figures 4a and 4b. Mean and S.E.M. of the air temperature versus exposure duration of (a) 14 male mice and (b) 14 female mice irradiation with a forward power of 5 W.

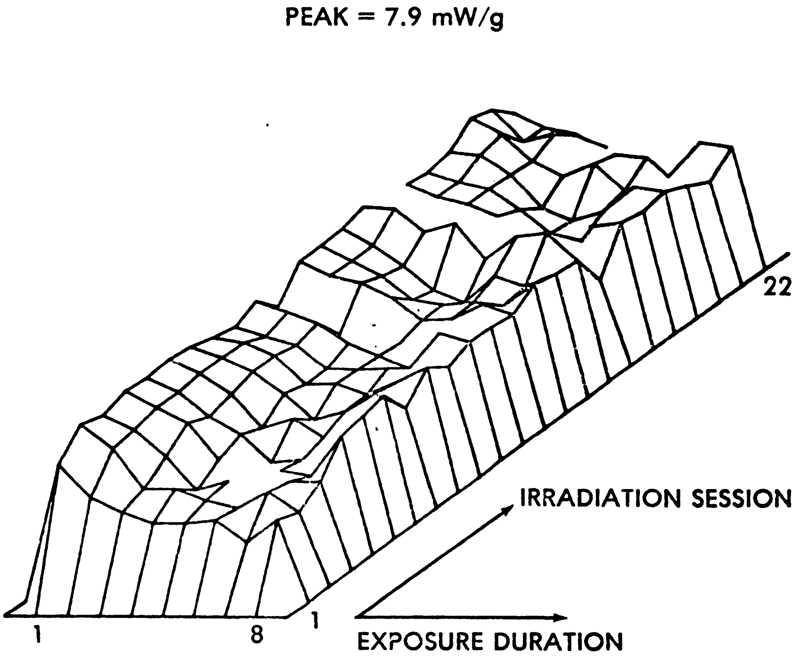

Figure 5a

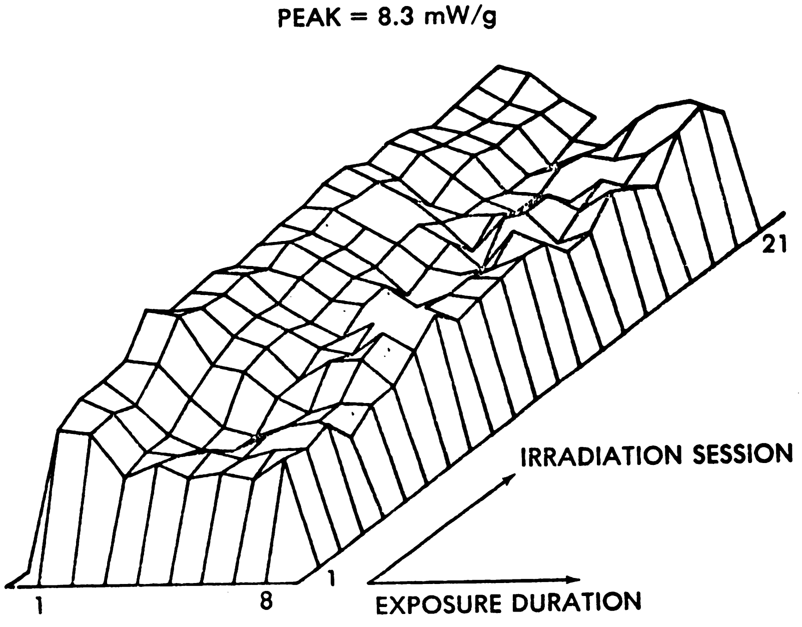

Figure 5b

Figures 5a and 5b. The average absorbed dose rate versus exposure duration, and irradiation session of (a) 14 male mice, and (b) 14 female mice irradiated with a forward power of 5 W.

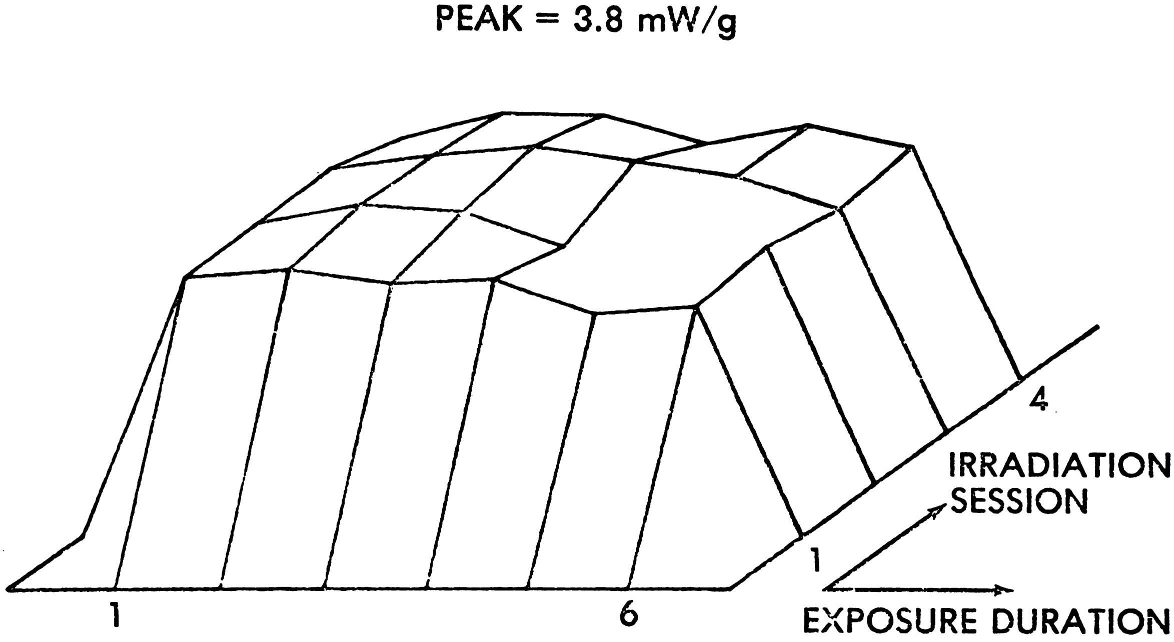

Figure 6. The average absorbed dose rate versus exposure duration, and irradiation session of 14 female mice irradiated with a forward power of 2.5 W.

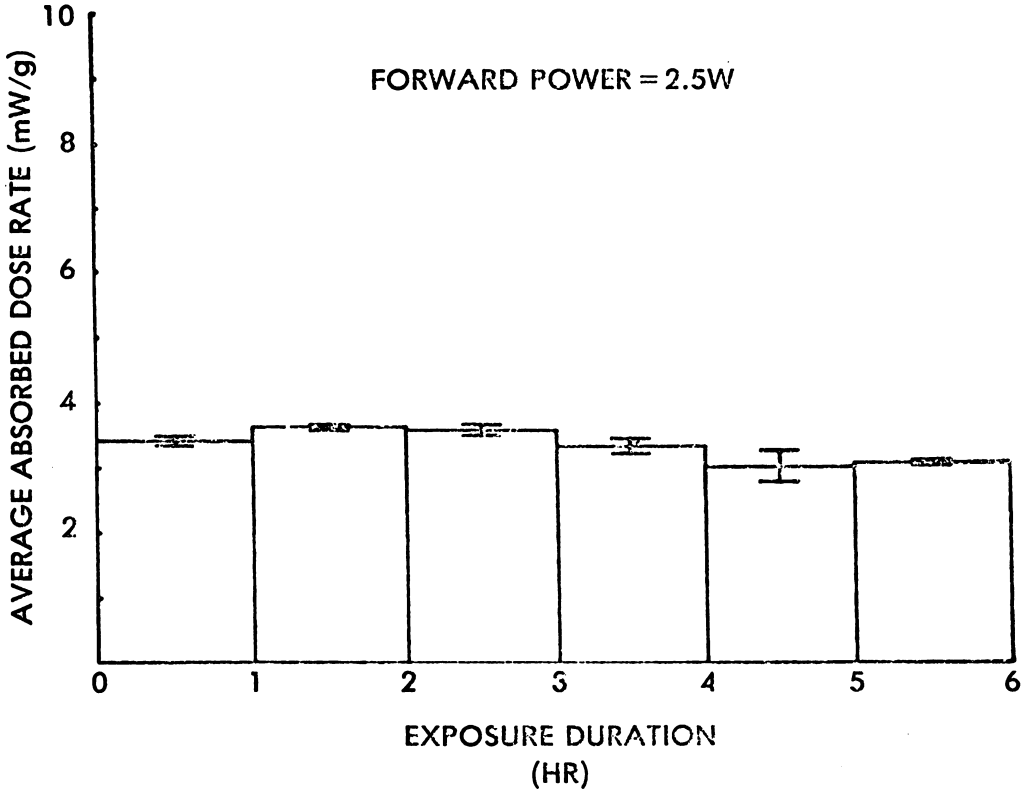

Figure 7. Mean and S.E.M. of the average absorbed dose rate versus exposure duration of 14 female mice irradiated with a forward power of 2.5 W.

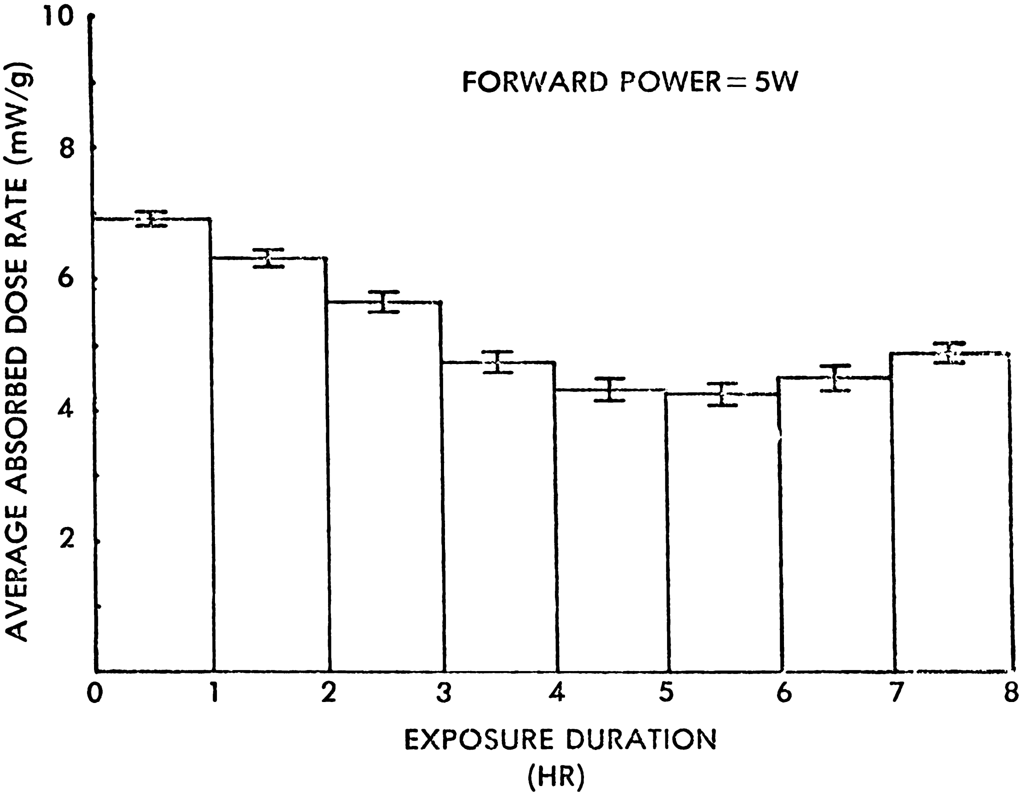

Figure 8a

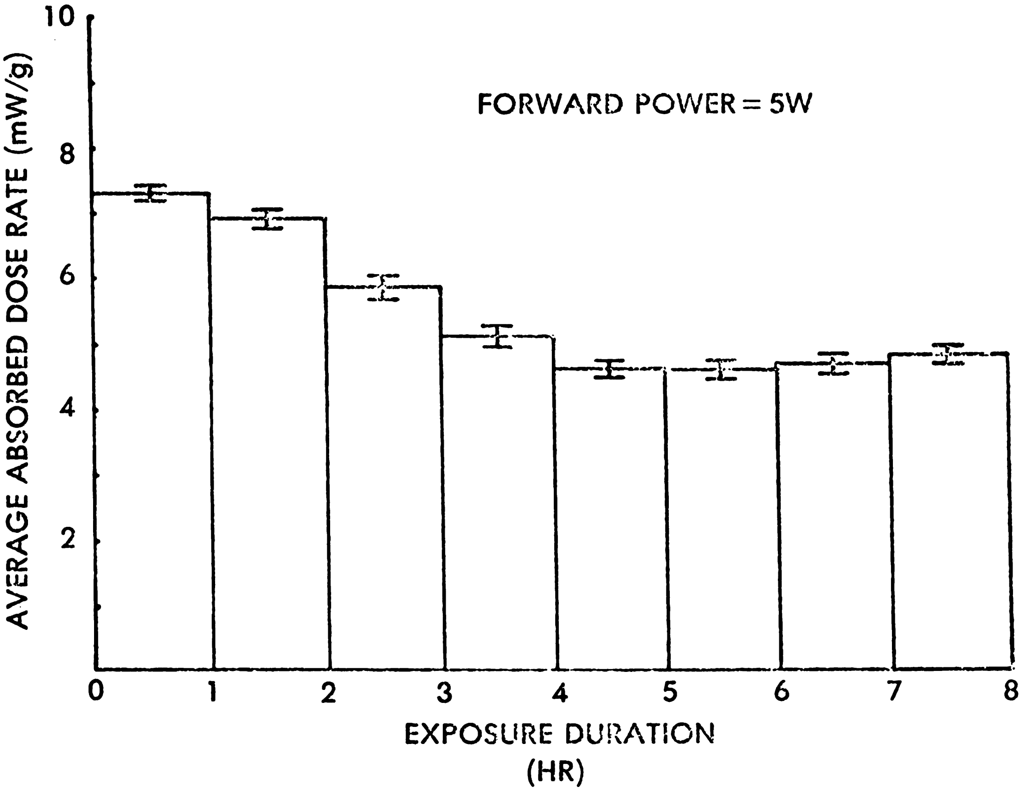

Figure 8b

Figures 8a and 8b. Mean and S.E.M. of the average absorbed dose rate versus irradiation session of (a) 14 male mice and (b) 14 female mice irradiated with a forward power of 5 W.

Figures 9(a) and (b) show the mean and S.E.M. of the average absorbed dose rate of the male and female groups of mice irradiated with a forward power of 5 W as a function of irradiation session, averaged over each session. For both the female and male group, the average absorbed dose rate seems lower in the first few sessions than in the rest of the sessions. One possible explanation for this change may be that the combined weight of each group was increasing with each session, thus affecting average absorbed dose rate. However, the lack of a perfect correlation with weight ($r = 0.41$ for males, and $r = 0.75$ for females) suggests that other factors may also be present. Comparison of air temperature and average absorbed dose rate versus irradiation session is shown in Figures 10(a) and 10(b) for the male and female group irradiated with a forward power of 5W. No definite correlation between air temperature and average absorbed dose rate can be found ($r = 0.503$ for the male group; $r = 0.553$ for the female group.) However, during the 15th irradiation session for the male group, the air temperature of approximately 21° C was lower than usual. The corresponding average absorbed dose rate of approximately 6.4 mW/g for the irradiation session was the highest observed during the whole experiment.

DISCUSSION

A reduction in microwave absorption during 8-hour irradiation sessions after the initial hour of each session was observed for both a female and a male group of mice exposed to 915 MHz CW radiation with a forward power of 5W resulting in an average absorbed dose rate of approximately 5 mW/g. This reduction was not observed for a forward power of 2.5 W which resulted in an average absorbed dose rate of approximately 3.4 mW/g. It should be pointed out that since the value 5 mW/g is an average over a whole group of mice, some mice in the group will receive more while others less than this mean value. Nevertheless, when one considers the reported higher levels of 23.6 - 28 mW/g which causes reduction of microwave energy absorption in the single exposure and short duration experiments, the results of the present experiment are interesting.

The general increase in absorption with increased irradiation sesssion cannot be simply explained by the physical parameter of weight increase of the mice alone. Additional experiments may be needed to explore the possible change in microwave energy absorption with repeated exposure.

The result of this investigation indicates that for a group of mice irradiated over a relatively long duration, the average absorbed dose rate may change with exposure duration; and the change depends on the intensity of the exposure field, and may also depend on large variations of the environmental temperature. This dynamic property of microwave energy absorption of irradiated animals may have serious implications on the determination of absorbed dosimetry and the corresponding interpretation of observed biological effects in animal irradiation apparatus where the rate of energy absorption is not monitored during the irradiation.

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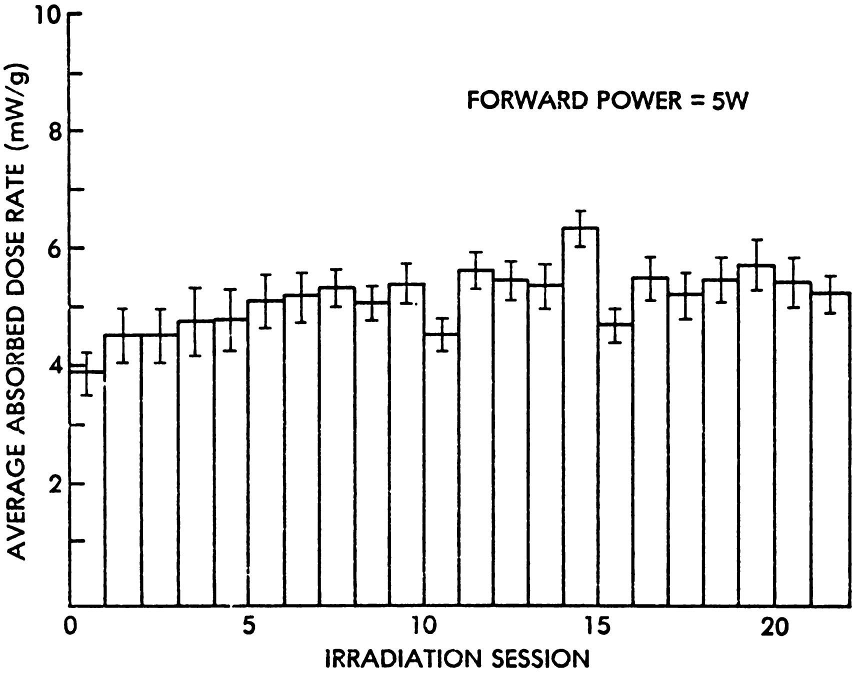

Figure 9a

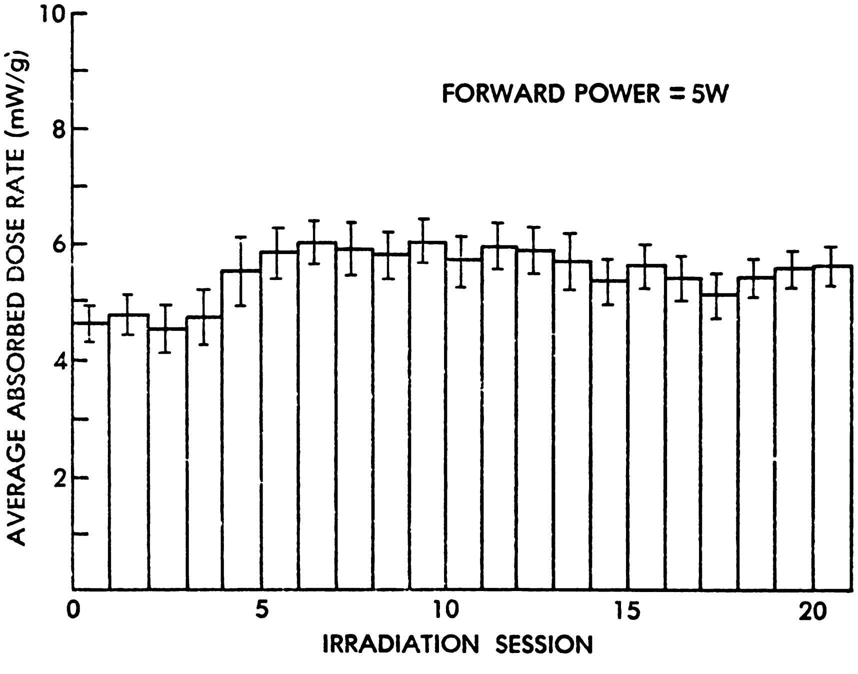

Figure 9b

Figures 9a and 9b. Mean and S.E.M. of the average absorbed dose rate versus exposure duration of (a) 14 male mice, and (b) 14 female mice irradiated with a forward power of 5 W.

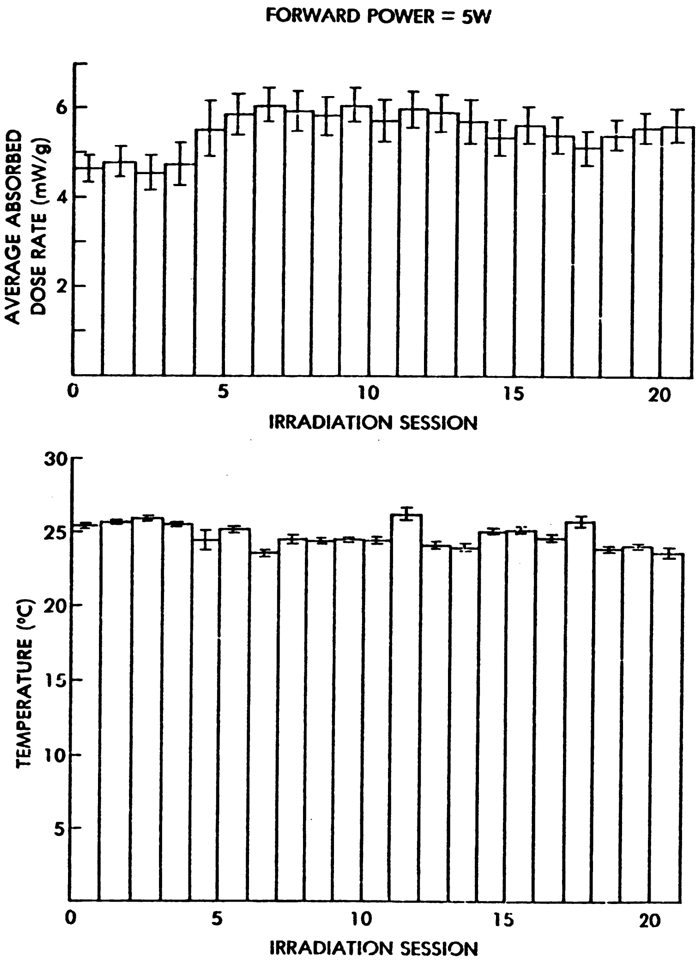

Figure 10a

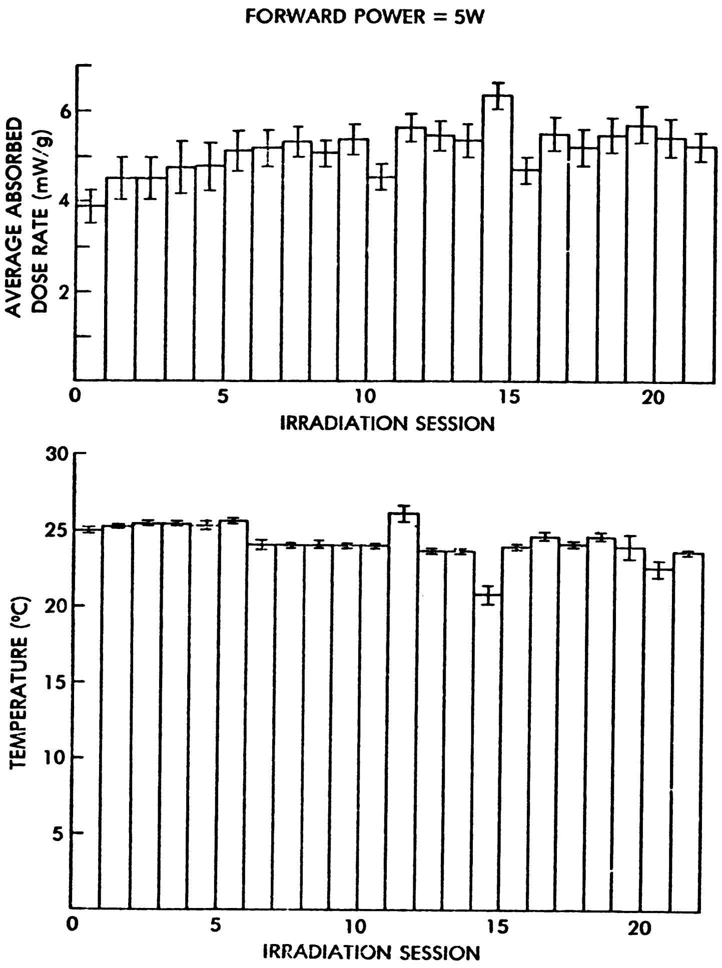

Figure 10b

Figures 10a and 10b. Mean and S.E.M. of the average absorbed dose rate and the air temperature versus irradiation session of (a) 14 male mice, and (b) 14 female mice irradiated with a forward power of 5 W.

2. Ho, H. S., and W. P. Edwards. Dose rate related effects on the oxygen consumption of mice during and after microwave irradiation. Presented at the URSI Annual Meeting, October 11-15, 1976, Amherst, Massachusetts.

OPEN DISCUSSION

DR. PHILLIPS: I would like to congratulate you on an excellent study. You have been getting into some of the questions many of us have been asking for quite a while. I have several comments and one question. The one question is: were you able, at any time, to observe the position or behavior of animals while they were being exposed?

DR. HO: Not during the irradiation because the waveguide is enclosed. However, the people doing the irradiation observed at times that when taken out, the animals would bunch up in a corner or something like that.

DR. PHILLIPS: These animals were not heavily restrained, is that correct?

DR. HO: Yes, that is correct. They were in a big box, as I mentioned.

DR. PHILLIPS: One interesting observation: we have done some single animal exposures and also recorded dynamically, looking at the position of animals, reflected power, or forward power in a cavity arranagement. We have found that as you go throughout a session, (this is at higher dose rates than you were using by the way, and this is also rats) that the absorption and reflection was heavily dependent upon the position the animal would assume. If the animal balled up, you would maximize absorption and minimize the reflected energy. As an animal became heated, he would typically go spread eagle, and usually you would get less absorption of energy. These results may be what your data is reflecting; particularly since you see it at the higher dose level and not the lower dose level. It would be interesting if you could in some manner with your system, be able to visualize the behavior of the animals.

DR. HO: We have done another study on the single animal exposure. As I have mentioned before, in the previous experiments, we have done the absorption with a relatively large holder so the animal can orient; and we see those reductions. Subsequently, we have also done another exposure, where we put the animals in a small holder where they cannot orient or effectively change their shape or orientation, so to speak. We do not get any reduction in absorption; we do get a higher metabolic rate or oxygen consumption rate.

DR. PHILLIPS: One last comment: in the mouse and rat, like in the human, the male tends to be larger than the female; and you will typically see that kind of data here.

DR. JUSTESEN: I was thinking about a way of getting at this question of whether there is some kind of ordinal artifact that I have been worrying about because of the data I presented yesterday. Have you envisioned or actually performed series without radiation to where you would radiate one group, for the first hour, the second group for the second hour, and the third group for the third hour? I will tell you what I am getting at. A novel situation calls forth a questionning reaction on the part of a mammal--they have to stir around, explore, and look to make sure things are safe if you have some kind of activity related artifact. Letting the animals thoroughly explore before they receive a bout of radiation, which in turn might be associated with some characteristic posture or kind of activity, would be a way of answering the question.

DR. HO: We have not done exactly what you said; however, as I mentioned in the preliminary experiment, we have used the lower level where we continue for six hours (that's pretty long) and we do not see that reduction. Of course, this is only for four sessions. Incidentally, we have actually done more irradiations with the higher level and we still see the same thing.

DR. JUSTESEN: One last comment: the reason why your data are very interesting, is because they might indicate that the rat has the capability that the human being does not. You weren't running 300 gram mice were you?

DR. HO: No. There were fourteen mice altogether.

DR. JUSTESEN: Right. I would like to get a hold of that strain. Dr. Carpenter might want to comment on this. It is my interpretatin that when God did the engineering, if I can put it that way, for the thermoreceptor system of the human being, He patterned it after the basic design of the eye. I say this in the sense that we have these exquisitely sensitive--in terms of vernier detail--receptors, rather the phobia. We have these light sensitive receptors that are wired in parallel; so that we sacrifice vernierity, localizability, in terms of behavior just for the sake of being able to respond to light, per se. It would appear as though thermoreceptors are wired the same way in human beings. The import seems to be that it does not matter where the heat is coming from, but "get the Hell out of there" seems to be the way that we are programmed.

If you take human beings and blindfold them, remove their shirts, cover their backs with carbon black so that you can get equal rates of absorption of mildly thermalizing energy--the average human being will be confused two thirds of the time, if you are putting the light say in the back of one arm or on the left side of the back. He will be confused to the extent that he will say that the temperature is being applied to the other arm. If the rat is being this exquisitely sensitive, this would be interesting from the standpoint of comparative psychologists and comparative physiologists.

DR. CARPENTER: I am not sure whether Dr. Justesen suggested that I comment on this engineering as the observer or as the designer of it. I am only an observer. If you are speaking otherwise, I feel flattered. I also have a question: when your several mice could romp around, if they romped the length of their Plexiglass holder, were they romping across the waveguide or the length of it?

DR. HO: Either way.

DR. CARPENTER: We observed some time ago in measuring the power density in a chamber in which there are eight mice, (they could not very easily pass each other) that what hit one mouse depended on where the other mice were. Consequently, as the mice moved, the reflectivity of the mouse changed the field so that another mouse sitting nearby might get quite a wallop; whereupon, he might move and this would change what all the other seven mice were getting. Does that sort of explain what is happening in your average absorbed dose rate--the average of the whole thing disregarding individual differences?

DR. HO: Exactly.

DR. CARPENTER: Now the mice themselves could conceivably increase the absorbed dose rate beyond what you would expect.

DR. HO: Yes, I mentioned in my conclusion that that is probably the case. What you mentioned, however, is something interesting in that it may take a group effort for the mice to reduce the absorbed dose rate; just one mouse will not reduce it.

DR. CARPENTER: Why don't you nail down your mice?

DR. HO: We have done so for individual mice, and they cannot reduce their absorption. We have done another experiment with a single mouse; if you put it in a small holder, it cannot reduce its absorption.

DR. CARPENTER: No, I mean several mice. If you have the same number of mice in the holder and cement their feet down, for example, so they can not run around; will you get a difference in the absorbed dose rate?

DR. HO: We have not done it; probably not.

DR. MICHAELSON: What Dr. Phillips said is very true; that is, the motion of any single mouse or multiple mice in the cavity will produce the same result. The mice are accomodating to whatever is bothering them; in this case it may be heat. There is no doubt about it, these animals will orient themselves or go placid. In other words, they stretch out to thermoregulate; that is very important to realize.

Another factor that has not been mentioned is that many people have observed (I think Dick Phillips has observed this, we have, the Russians have, and I believe Gordon has) that on repeated exposures over days you do have the factor of physiologic adaptation. Also, in the eight hour exposure, there is a very important factor that has not been mentioned--circadian periodicities of many body functions. This could easily relate to some of the responses you see which, in fact, alter the absorbed dose. It is an extremely important factor to take into consideration. I do not think that it takes heroic efforts or a multi-million dollar study to pin these things down. I think you have a lot of the information available to you now. I am sure you, as an engineer, could figure out a system to observe that animal and yet not let him see you. This is a very important consideration.

THE DESCRIPTION OF A SYSTEM TO IRRADIATE CELLS IN CULTURE WITH MICROWAVES

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ABSTRACT

The need for identifying the biomolecular systems affected by microwave is discussed. A system for irradiating cultured cells or other biological samples is described. A formalism for treating the biological response in this system is introduced and analyzed. The effect of the temperature distribution within the sample on the biological response is illustrated for glucose efflux from red blood cells and ATPase activity.

INTRODUCTION

Extensive experimental studies on the biological effect of microwave radiation have been performed over the past 30 years (1). Theoretical analysis of the underlying mechanisms have been much less extensive and have been limited primarily to consideration of macroscopic physical characteristics of biological materials.

The absorption of energy on the molecular level from electric and magnetic fields has also been extensively studied, and the quantitative theory that relates the frequency of the applied fields to absorbtion of energy and perturbations in molecular structure is well developed (2). Further, one of the main thrusts of modern molecular biology is to elucidate the relationship between molecular structure and biological function. General qualitative mechanisms for the absorption of microwave radiation and the induction of functional changes in biological molecules have been proposed (3,4,5), but quantitative discussion of these models is not possible until a specific biomolecular system is identified, that is, known to be affected by microwave radiation.

It is clear that the macroscopic dielectric properties of biological systems can be understood in terms of the molecular structure of water and the amount of free (and bound) water present in the system. The interaction of the field with numerically minor but functionally significant biomolecules will have negligible input into the dielectric constant but may have important functional implications.

The purpose of this paper is to describe and present a basis for quantitative analysis of a system for irradiating cell in culture or a biomolecular preparation and measuring a specific molecular function during irradiation. The ubiquitous presence of water in a biological active preparation will dominate the rate of energy absorption.

In cellular and subcellular experiments, the thermoregulatory mechanism of the animal is not available and thermal effects will be seen at relatively small absorbed energies. This can be partially controlled by external cooling, but there will always be a temperature distribution within a sample of finite size that cannot be duplicated by any addition or subtraction of heat through the surface. In order to interpret the results of this type of experiment, it is critical to know the thermal response of the measured function within the temperature range of the sample.

It is important at this point to have a careful definition of thermal response. A response, if it is a result of the collisonal transfer of energy from one molecule to another, is thermal. Temperature changes may be associated with, though unrelated to, responses that are not thermal in origin. Moreover, responses not associated with macroscopic temperature changes may be thermal in origin due to microscopic heating or changes in state due to the transfer of kinetic energy. The thermal response of a system can be measured by adding kinetic energy through its surface and measuring the response after the system has reached thermal equilibrium.

The system to be described in this paper enables one to irradiate small biological preparations in conditions that allow for the prediction of the temperature distribution within the preparation.

MATERIALS AND METHODS

The biological samples to be studies are kept in Teflon tubes shown in figure 1. These tubes can contain up to 1.6 ml and cells could be cultured indefinitely in them. For an exposure to microwave radiation, the surface temperature of the tube is controlled by inserting it into a jacket through which a coolant is pumped. Figure 2 is an assembly diagram of the tube and jacket. Dodecane, a liquid alkane with $\varepsilon' = 2.10$ and $\varepsilon'' < 10^{-4}$ (6) (for Teflon $\varepsilon' = 2.10$ and $\varepsilon'' < 10^{-3}$) is used as the coolant. The choice of dodecane as a coolant is based on the similarity of its dielectric properties to Teflon and trade-off between viscosity, vapor pressure, and heat capacity. The resulting composite cylinder and central cylinder containing the biological sample are small enough that at 2.45 GHz they act as absorbers and do not propagate the field along the cylindrical axis.

The use of the coolant also provides a method for measuring the power absorbed by the sample. This is accomplished by comparing the resistances of (matched) thermistors (Fenwal Electronics, UUT511J1) placed at the input and output ports of the jacket, using a Wheatstone Bridge with a Hewlett Packard 419A DC null meter. The match of the thermistors is not good enough for our purposes and a discussion of the corrections required appears in a later section. The coolant is controlled in a HAAKE FKP circulator bath. While it might be possible to use this system for a far-field exposure, exposing in a waveguide allows us to have the thermistors closer to the sample and yet outside the field, decreases the distance the coolant flows between the bath and the sample, and allows more complete definition of the field in the sample.

The waveguide irradiation system is shown schematically in figure 3. The bidirectional coupler provides a means of measuring both the incident and reflected microwave power. The tuning section consists of two movable blocks and allows matching of the inductive and capacitive discontinuities that occur in the system as well as those introduced by insertion of the biological samples. By adjusting the sliding short and the tuner, it is possible to match a sample into the system so that almost all the incident energy is absorbed and very little energy is reflected. A power reflection coefficient (reflected power/incident power) of 10^{-3} has been measured for rabbit blood samples inserted into the sample chamber. The sliding short allows us to have an electric field maximum at the center of the sample.

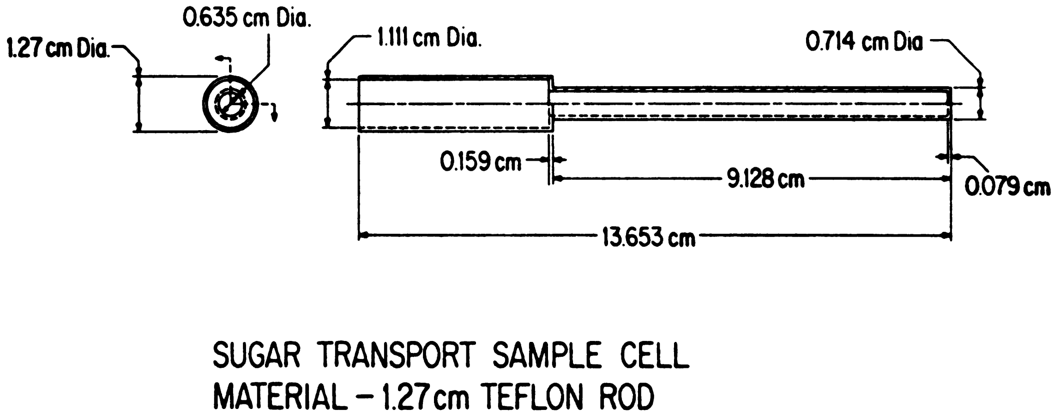
0.635 cm Dia.
1.27 cm Dia.
1.111 cm Dia.
0.714 cm Dia
0.159 cm
9.128 cm
0.079 cm
13.653 cm
SUGAR TRANSPORT SAMPLE CELL
MATERIAL - 1.27cm TEFLON ROD

Figure 1. Sample Cell

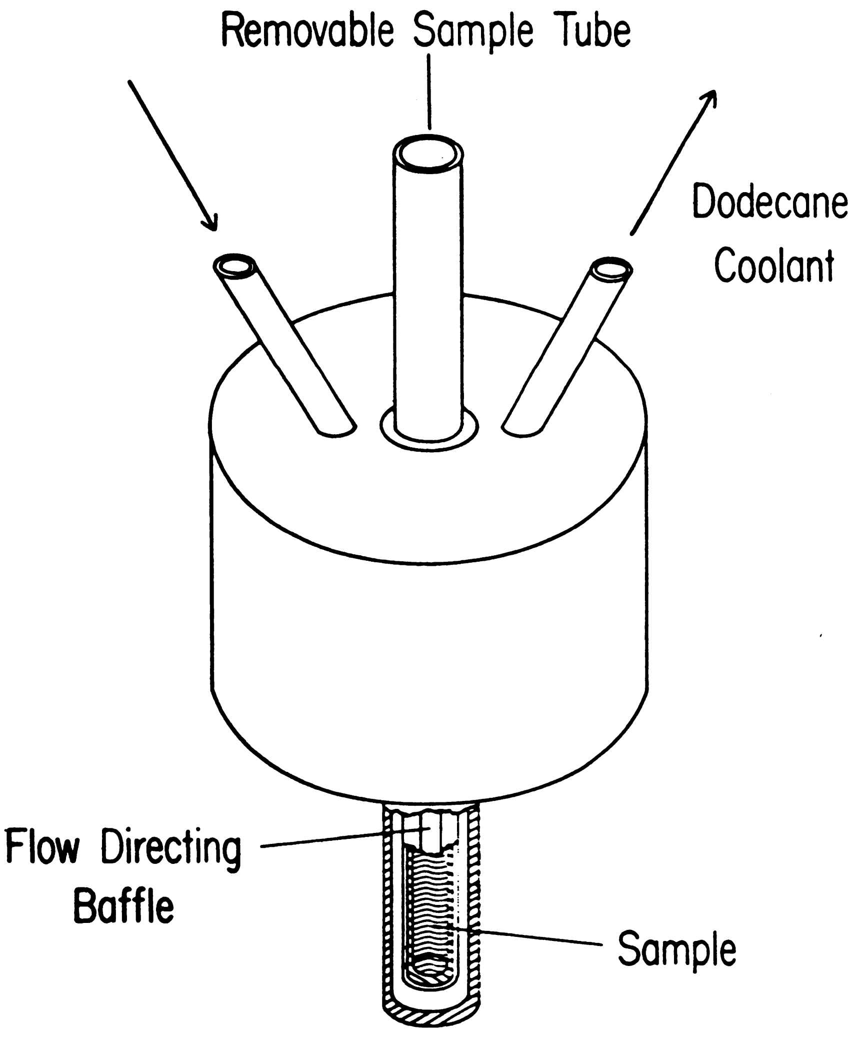
Removable Sample Tube
Dodecane
Coolant
Flow Directing
Baffle
Sample

Figure 2. Assembly diagram of sample compartment and coolant.

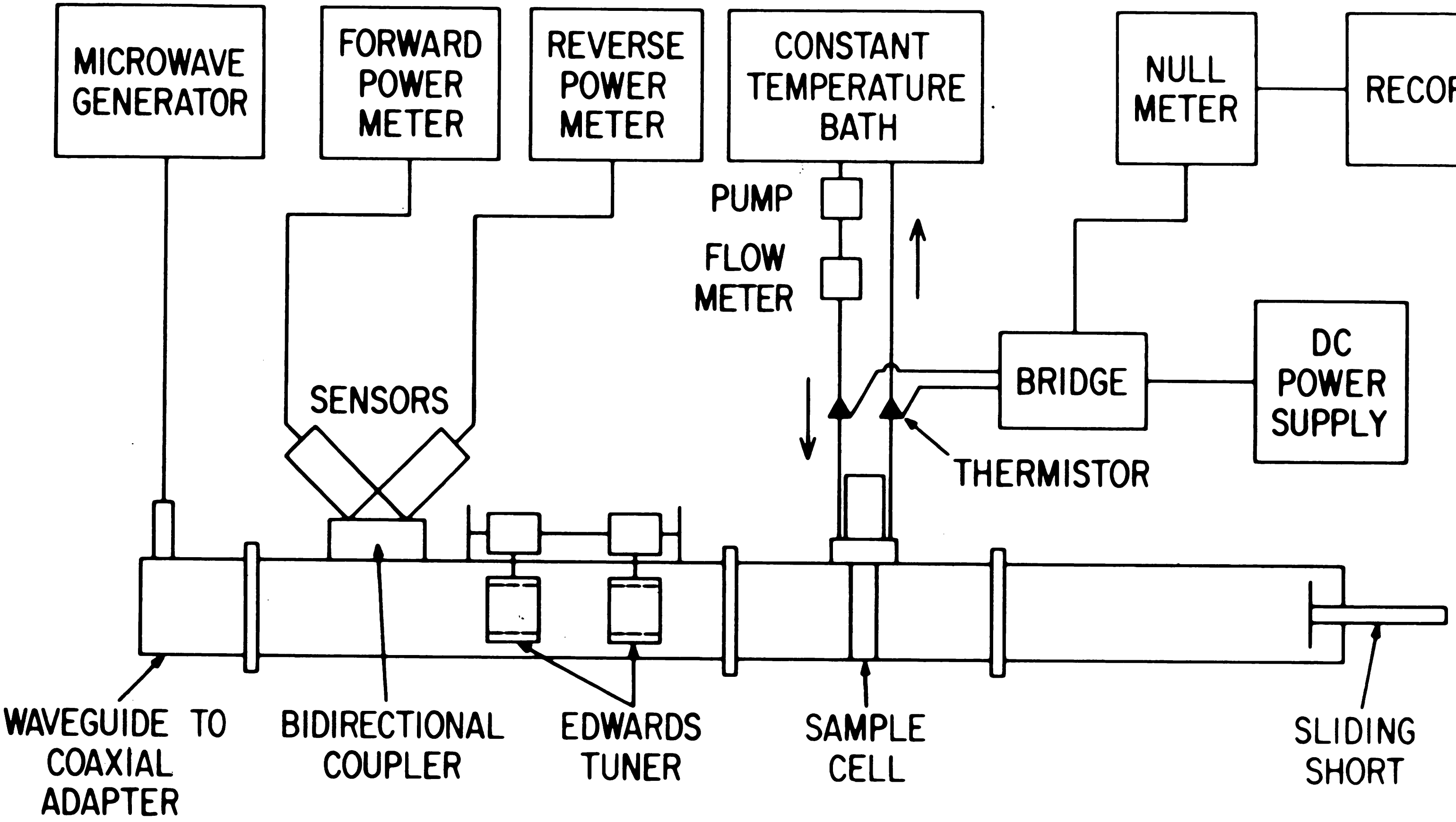
MICROWAVE GENERATOR
FORWARD POWER METER
REVERSE POWER METER
CONSTANT TEMPERATURE BATH
NULL METER
RECORDER
PUMP
FLOW METER
BRIDGE
DC POWER SUPPLY
SENSORS
THERMISTOR
WAVEGUIDE TO COAXIAL ADAPTER
BIDIRECTIONAL COUPLER
EDWARDS TUNER
SAMPLE CELL
SLIDING SHORT

Figure 3. Schematic diagram of exposure system.

RESULTS AND DISCUSSION

The purpose of the apparatus just described is to yield a response function R_{θ^o} (P,t) that is dependent on the bath temperature (θ^o), the rate at which energy is absorbed from the field (P) and the time from the beginning of the exposure (t). The time needed to reach equilibrium in the sample tube has been measured using both the bridge and a tele-thermometer (turning the field off after short times) and found to be about 90 sec. For the remainder of this discussion we assume that the biological sample is irradiated for times long with respect to 90 sec. and

$$\therefore \int R_{\theta_0} (P,t)\, dt = R_{\theta_0} (P)\, (t_f - t_i) \qquad (1)$$

$(t_f - t_i)$ is the duration of the exposure. The rate of energy absorption from the field and energy lost by the sample to the coolant are equal and independent of time.

$$R_{\theta_0} (P) = \int S\, (\theta, \eta)\, d\vec{r} \qquad (2)$$

Where the integral is over the entire sample, $S(\theta,\eta)$ is the point response function dependent on the temperature (σ) at that point and perhaps on the net effect of other factors (η) which at this time may not have been fully identified, and, in any case, cannot as yet be quantitated. For example, if the response (R) is the cleaving of a small molecule by an enzyme, then $S(\vec{r}_a)\, \Delta\vec{r}_a$ is the amount of the small molecule cleaved in the volume $\Delta\vec{r}_a$ centered on r_a where $\Delta\vec{r}_a$ is defined as small with respect to macorscopic sample volumes but large enough to contain enough molecules (be they enzyme, substrate or water) so that a temperature can be defined. Obviously S $(\vec{r}_a)$ depends on the temperature (θ_a) at r_a but it also depends on the amount of substrate at r_a (η_a). θ_a depends on only macroscopic parameters, P (the rate of energy absorbed from the field), geometric properties of the sample and sample holder and physical properties of the materials involved (dielectric constants, heat conductivities, ...). It is less clear what η depends on. It is worth noting, however, that if η is a function of P, that does not necessarily imply a nonthermal effect. For instance, if the response we are maeasuring is the product of an enzyme embedded in the cell membrane and the enzyme activity is dependent on the structure of the lipids that surround it, then the melting or freezing of those lipids will have a large effect on the response in a way that is strictly thermal but not dependent on a change in temperature.

It is also important to note that many of the responses are not linear in θ so that $S(\overline{\theta}) \neq \overline{S(\theta)}$. Therefore it is critical to know how the biological response measured depends on temperature and what is the temperature distribution within the irradiated sample.

Measuring the rate of energy absorption in the sample by comparing the resistance of commercially available (matched) thermistors in the input and output port of the coolant necessitates consideration of the differences in the thermistor characteristics. A description of the corrections necessitates consideration of the differences in the thermistor characteristics. A description of the corrections necessary follows. In the wheatstone bridge at zero current through the null meter,

$$\frac{r_i}{r_e} = \frac{r_d}{r_f} = \frac{A_i\, e^{\beta_i \left(\frac{1}{T_i} - \frac{1}{T_o}\right)}}{A_e\, e^{\beta_e \left(\frac{1}{T_e} - \frac{1}{T_o}\right)}} = \frac{A_i}{A_e}\, e^{\beta_i \left(\frac{1}{T_i} - \frac{1}{T_o}\right) - \beta_e \left(\frac{1}{T_e} - \frac{1}{T_o}\right)} \qquad (3)$$

where r_i is the resistance of the thermistor at the input port
r_e is the resistance of the thermistor at the exit port

r_f is the resistance of a fixed resistor
r_d is the resistance of an adjustable resistor

and $1 >> \Delta\beta/\beta > \Delta T/T$ where

$$\beta = \beta_i + \Delta\beta, \ T = T_e = T_i + \Delta T$$

Taking the ln of both sides gives

$$\ln (r_d/r_f) = K + \Delta\beta/T_0 + \beta/T - \beta + \Delta\beta/T + \Delta T \tag{4}$$

where $K = \ln A_i/A_e$ expanding in power of $\Delta T/T$ and ignoring factors of $(\Delta T/t)^2$ higher powers and then solving for ΔT yields,

$$\Delta T = \frac{T^2 \ln (\frac{r_d}{r_b}) - KT^2 - \frac{\Delta\beta T^2}{T_o} + \Delta\beta T}{\beta + \Delta\beta} \tag{5}$$

The rate of energy absorption is proportional to ΔT and the portionality constant is equal to the specific heat of the dodecane multiplied by the flow rate. It is better to calibrate Equation (5) for rate of energy absorption by dissipating known amounts of power not from microwave sources.

$$P = c \bullet V \bullet \Delta T = C \Delta T \tag{6}$$

There are still unknown parameters in Equation (5). They can be quantitated by measuring $\ln (r_d/r_f)$ as a function of coolant temperature with no absorbed energy, $\Delta T = 0$, shown in figure 4. $\Delta\beta$ is assumed to have a linear temperature dependence and it is found that this gives a better fit to the data than $\Delta\beta$ a constant. $\Delta\beta$ is found to be much less temperature-dependent than β is. Figure 5 gives P input as a function of $\ln (r_d/r_f)$ measured at constant temperature. The rate of absorbed energy measured by Equations (5) and (6) agrees with the forward power measured in the tuning section to .1 db.

The temperature distribution within the sample can be calculated from Poissons equation,

$$\nabla^2\theta (r,\phi,Z) = -1/K \ q (r,\phi,Z) \tag{7}$$

where K is the heat conductivity of the biological material in the cylinder, $q (r,\phi,Z)$ is the heat generated within the sample, per unit time, and

$\int q(r,\phi,Z) \ 2\pi r \ dr \ d\phi \ dZ = P$ where the integral is over the tube and P is the rate of energy absorption. (8)

Methods for calculating the electric field inside concentric cylinders in a rectangular wave guide are in the literature (7,8). If we assume that the heat produced in the sample is a result of the electric field, then

$$q(r,\phi,Z) = \Sigma_{nm} A_{nm} J_n (k_s r) J_m (k_s r) \text{Cos } n \phi \text{ Cos } m \phi \tag{9}$$

where A_{nm} is a constant dependent on the dielectric properties of the biological material (water) and the dielectric and geometric properties of the cylinders. J_n and J_m are ordinary bessel function. In theory, Equation (7) can be solved using the Green's function for a cylinder with the fixed temperature ($\theta = \theta^0$) on the surface as the boundary condition. Rather than solve Equation (7) numerically, it is more illustrative to consider some extreme cases: first, where the q is a constant, and second, where q is the $A\text{Cos}^2 2\pi r/\lambda$. These will

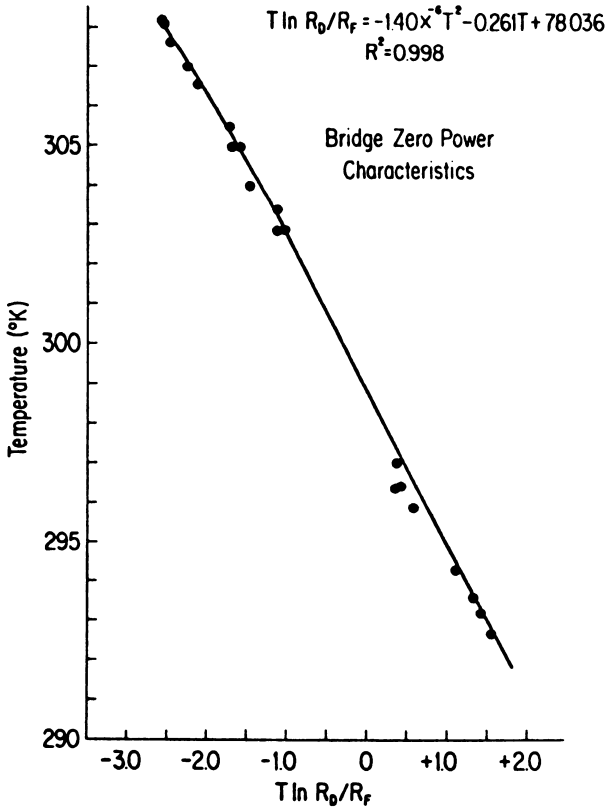

Figure 4. Experimental evaluation of bridge constants with $\Delta T = 0$.

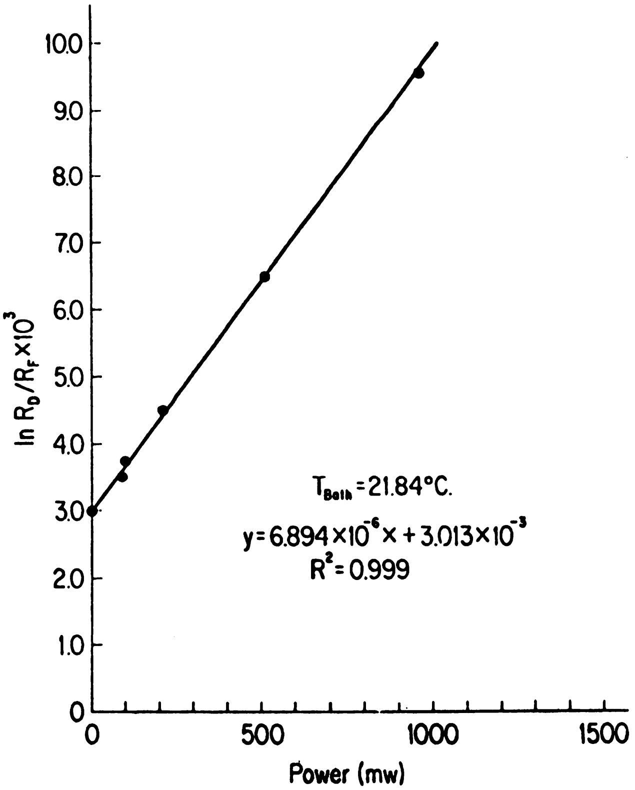

Figure 5. A sample calibration of ln (R_0/R_b) vs. power at a fixed temperature.

give an upper and lower bound to the difference between the maximum temperature within the cylinder and the surface temperature. The first case assumes the rate of energy absorption is uniform over the cell. The second case is the field profile in the Z-Y plane rotated through 180° to give a 3-dimension field (see figure 6).

$$\Delta\theta\ (\vec{r}) = \theta - \theta^{o} = -\ P\ r^2/4K\ (\pi a^2\ h) \text{ for Case 1.} \qquad (10a)$$

$$= -\ P/K\ (a\ {}^2\pi h)\ \ (r^2/4 + \lambda^2/8\pi 2\ \sin^2\ 2\pi r/\lambda) \text{ for Case 2.} \qquad (10b)$$

where a $^2\pi h$ is the volume of the cylinder.

For an absorption rate of 400 mW (266 mW/cm^3) and the geometry of our apparatus, the maximum temperature difference within the sample is 1.1° C for Case 1 and 1.9° for Case 2.

If the biological response we are measuring is the flux velocity of an ion or small molecule into or out of cells in culture, then our point response S(r) = S [θ (r)], if S(r) is only a function of temperature, is

$$S(\theta) = c_1 e^{-c_2/\theta} \qquad (11)$$

where c_1 and c_2 are biological parameters that can be determined. For many different cells and small molecules values of c_1, c_2 are in the literature.

$$S(\vec{r}) = c_1 e^{-c_2/[\theta_0 - \Delta\theta(r)]} \qquad (12)$$

For Case 1, if $\Delta\theta/\theta^o$ is small, and biological response is only a result of temperatue change, the response from Equation (2) is

$$R_{\theta_0}\ (\sigma) = C\ (h, c_1, c_2, \theta_0) \left[\frac{e^{\left(\frac{c_2 a^2 \sigma}{4K}\right)} - 1}{\sigma} \right] \qquad (13)$$

to powers first order in $\Delta\theta/\theta^o$, where C is just a constant determined by the geometric and biological parameters of the system and σ is the rate of energy absorption per unit volume ($P/a^2\ \pi h$). For Case 2, we do not have a comparable closed form; and the thermal response function must be numerically integrated. This analysis allows for the separation of responses due to temperature distribution within the sample from responses due to other factors (η).

The effect of these temperature distributions within the sample on the response function depends critically on the response we are considering. For glucose efflux from human erythrocytes at a bath temperature of 35° C and an energy absorption rate of 200 mW/cm^3 there will be a 12 percent increase in Case 1 and 20 percent increase in Case 2 from the expected efflux at the bath temperatue, using our geometry and biological constants given in the literature (9). For Na-K ATPase activity for the same irradiation conditions using constants from the literature (10) there will be a 40 percent increase in the minimum case and 70 percent increase in the maximum case.

CONCLUSION

A system has been described for the exposure of a biological preparation to microwaves in a temperature-controlled environment. The temperature control apparatus can be used to measure the rate of energy absorption by the

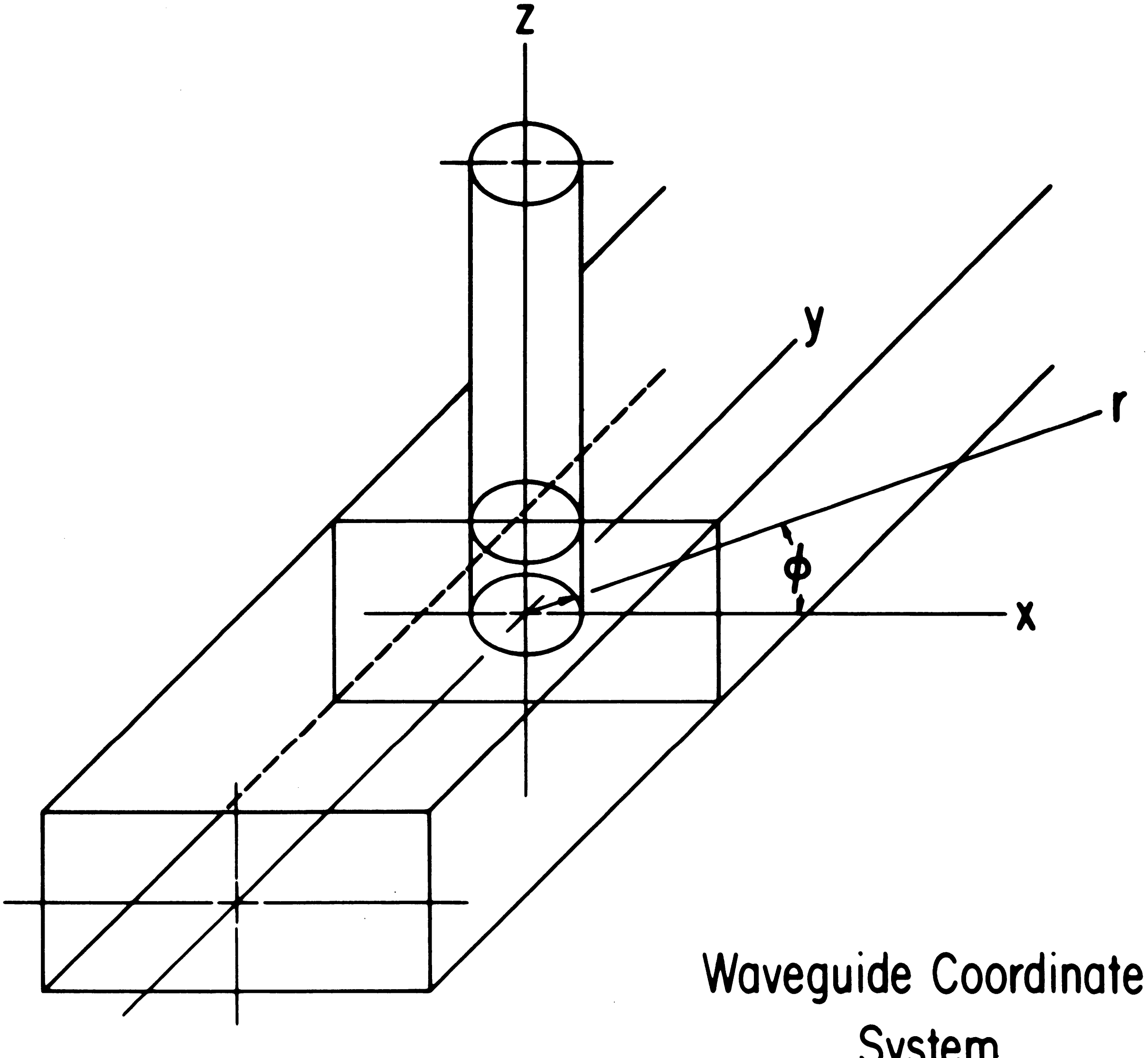
z
y
r
φ
x
Waveguide Coordinate System

Figure 6. The relationship between the waveguide coordinate system and the cylindrical system.

preparation. The temperature variation within the sample is known from theoretical consideration. This variation can have a significant effect on the biological response measured. For glucose efflux from human red blood cells for an energy absorption rate of 200 mW/cm^3, the temperatue variation was found to cause a (12-20) percent increase in biological response. For ATPase activity under the same conditions, the temperature variation was found to case a (40-70) percent increase in biological response. The effect of the temperature variation within the sample is highly dependent on the type of biological response being investigated and must be taken into account or else it will lead to erroneous results.

The paticular responses that were chosen to illustrate this point were chosen because there is evidence in the literature that they follow Equation (11) (as many other biological processes do). If the response under study did not follow Equation (11), but had a discontinuous jump at a temperatue within the temperature distribution of the sample, the effect could be much greater. This would be the case if there was a microscopic phase change in molecular structures relevant to the biological response within that temperature range.

ACKNOWLEDGEMENT

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OPEN DISCUSSION

DR. KANTOR: Do you have a transverse electric mode impinging on your culture cell? Is that in the waveguide?

DR. RABINOWITZ: Yes, we are using a Transverse Electric TE(0,1) mode in the waveguide.

DR. KANTOR: Are you aware of the fact that if you use the Teflon loaded waveguide, you would have a transverse electromagnetic mode in the center. As a matter of fact, I used this as a design for contact applicators. It might be useful to see what happens to your cell structure on the transverse electromagnetic excitation and compare it with your results of a transverse electric mode. I presume, although I am not familiar with the scale of your cell structure order versus wavelengths, that certainly if you use a transverse electromagnetic mode you would remove any ambiguity as far as size is concerned.

DR. RABINOWITZ: Are you talking about along the Teflon loaded waveguide?

DR. KANTOR: I am just saying that if you lower the waveguide with Teflon slabs on both sides, in the center you would have a transverse electric mode propagating between the two slabs. Further, I am saying that for the purpose of studying your very sophisticated type of analysis, it might be worthwhile to look into a simpler source--a radiation source rather than a transverse electric mode.

DR. BOCKSTAHLER: I am interested in your sample holder. An enzyme solution will continue to move around, but I am wondering about the cells. The cells that I work with would tend, if you have them in test tubes, to sediment just by gravity and drift down to the bottom. Do you have such a problem with your cell holder?

DR. RABINOWITZ: There is sedimentation, though not much. We try things like stirring, which adds a lot of power to the system, to avoid sedimentation. You also can measure the power you are putting in by stirring, and you will see noticeable changes.

DR. BOCKSTAHLER: Lastly, I was thinking in terms of the previous talk wherein the mice were running around influencing the field that each would get. Would the cells be too small so that there would be no effect from one cell?

DR. RABINOWITZ: Yes, I believe so.

DR. ROSENTHAL: I believe I missed your presentation covering a bio-response. What was the response you were looking for?

DR. RABINOWITZ: The mathematics is a general thing, but the particular response that we have looked at is glucose efflux from erythrocytes.

DR. ROSENTHAL: How did you pick that up--was it during the experiment?

DR. RABINOWITZ: During the experiment, yes.

DR. ROSENTHAL: How did you do that?

DR. RABINOWITZ: We used radioactive glucose.

DR. ANDERSEN: I was bothered throughout your talk by your continuous reference to your measurement of absorbed power. A point of fact, you have not measured absorbed power; you have measured the energy transferred from your sample to your coolant.

DR. RABINOWITZ: Yes.

DR. ANDERSEN: There are assumptions in that energy transfer. I was wondering what tests you have made of those assumptions as far as energy transfer from the sample to the coolant?

DR. RABINOWITZ: I really have made no test except to check if it is at equilibrium. If the energy in is equal to the energy out after those 90 seconds, I do not see why the two should be different.

DR. ANDERSEN: That would be true; but I have not seen the evidence that, in fact, you have reached equilibrium.

DR. RABINOWITZ: After 90 seconds?

DR. ANDERSEN: That is an assumption.

DR. RABINOWITZ: We have measured the temperature at times up to 90 seconds and after 90 seconds, and the temperature certainly remains constant.

DR. ANDERSEN: You measured the temperature of what?

DR. RABINOWITZ: The difference between the input and the output port after 90 seconds.

DR. ANDERSEN: I maintain that what you have measured is indeed the steady state temperature of the coolant, which may or may not reflect the exact nature of what is going on in the sample.

DR. CHEUNG: You have a coaxial type of cooling arrangement--what is the distance of the sample holding compartment? Have you measured in between to see whether you do get a higher temperature in the center as compared to the two walls?

DR. RABINOWITZ: Yes, that is what I did. The sample holding compartment has a 1/8-inch diameter. We can measure an average temperature. We do not have the equipment anywhere for measuring a few temperatures within there; and although we can get different temperatures, we cannot really get a temperature distribution by measuring in there. Theoretically we have calculated a maximum temperature difference of 1.9° from the center to the wall, and in practice we have measured a 1.2° temperature rise.

DR. JUSTESEN: There is a calculated temperature difference, as you have indicated, between the center and the outside of approximately 2°. Are you saying that your absorbed power measurement as indicated by the thermistor measurements are strictly an average, and you are not trying to reflect with that value any power distribtion within your sample holder?

DR. RABINOWITZ: No, not at all. It cannot do that.

DR. ROSENTHAL: Why didn't you measure the power directly; in other words, why didn't you put a load at the end? You can have directional couplers on both sides if you want or there are other ways of measuring power, and then you can measure the power absorbed directly rather than through a secondary means.

DR. RABINOWITZ: We measure forward reflected power. The absorbed power that we measure calorimetrically, at the sample chamber, indicates that better than 99 percent of the incident energy is absorbed in the sample. Waveguide losses and mismatches are responsible for very little absorption. We also felt that measuring transmitted, reflected, and incident power introduced three sources of error in calculating absorbed power.

DR. BRADLEY: If I understood you, you said that you have attributed the biological effects to the difference between 35° in the center of your tube and 37° at the periphery. Is that correct?

DR. RABINOWITZ: Yes.

DR. BRADLEY: Have you done an experiment of putting the cells in a 35° and 37° water bath and seeing whether you get the same sort of differences in the efflux of sugar?

DR. RABINOWITZ: Yes, that is what I was trying to say. You need that information first. It is not as though there is just 2° difference in temperature; there is a whole spectrum of temperatures in between.

DR. BRADLEY: I do not understand how you are going to get from 37° to 35° except by going down temperature gradient.

DR. RABINOWITZ: Right. That is how you get from 37° to 35°.

DR. BRADLEY: So the maximum effect would be the difference between 35° and 37°.

DR. RABINOWITZ: Right, that would be the maximum effect.

DR. BRADLEY: The 2° temperature difference makes more than a 40 percent difference?

DR. RABNOWITZ: If we assume it is all 2° higher, yes; but that is greater than the difference observed.

MR. HARRISON: Do you remember what the forward power was that causes 2° rise?

DR. RABINOWITZ: The forward power was 200 milliwatts per cubic centimeter.

DR. SEAMAN: I work with a similar radiation system not using waveguide but rectangular coaxial stripline. I think your assumption that the temperature is symetric around the axis of the sample is not correct.

DR. RABINOWITZ: I think that that is an extreme case. I said that we are not going to solve this thing exactly just because I want to get an idea of what is going on, I do not want to do that. I simply took the idea that the temperature is symetric around the axis as one possible case. Is it extremely different than that; do you think that is a very bad assumption?

DR. SEAMAN: It is not; however, it is an assumption, and it is only an assumption. You would have to measure it to get an idea, because it depends upon the absorption at that frequency as well as the thermal diffusion between the coolant and the sample.

DR. RABINOWITZ: Right.

DR. CHEUNG: It seems to me that if you increase a flow rate or your coolant rate, you should be able to reduce a temperature gradient of at least two degrees.

DR. RABINOWITZ: No.

DR. CHEUNG: You cannot?

DR. RABINOWITZ: We cannot do that because we are taking out all the power that is going in at that point. If we are assuming that we are at equilibrium now you need a temperature gradient to lose heat.

DR. CHEUNG: It depends on how thin your sample is; there is a constant for one eighth of an inch.

DR. RABINOWITZ: But also for the thickness of the sample itself, you need a gradient to cross the sample in order to lose heat.

DR. CHEUNG: Couldn't I take out a little more?

DR. RABINOWITZ: This is taking it out as quickly as it is going to come out. It is not the flow of the dodecane that is important; it is the flow of the heat through the sample that we must be concerned with.

NEW TYPES OF MICROWAVE DIATHERMY APPLICATORS -- COMPARISON OF PERFORMANCE WITH CONVENTIONAL TYPES

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Bureau of Radiological Health, FDA

ABSTRACT

Heating patterns induced in planar phantoms of simulated fat and muscle by direct contact applicators -- the new types -- and by spaced applicators -- the conventional types -- are compared. The new applicators provide for more uniform heating in the center of the temperature profiles than the conventional types presently in general clinical use. Unwanted irradiation of operator and unprescribed tissue of patient is much more easily controllable with direct contact applicators than with spaced applicators. Electric field measurements show that the leakage radiation of a commercially available direct contact applicator is less than 5 mW/cm^2 per 100 Watts of forward power at a spacing of 5 cm from the aperture-phantom boundary while scattered field levels for loaded spaced applicator can typically be 30 mW/cm^2 per 100 Watts of forward power at a distance of 13 cm in front of the aperture.

INTRODUCTION

The conventional types of applicators presently in clinical use for external treatments in microwave diathermy operating at 2.45 GHz are spaced applicators for high power therapy and direct contact applicators for low power therapy. Spaced applicators can impinge scattered as well as radiated energy on the operator or the unprescribed tissue of the patient. Direct contact applicators are inherently safer because they reduce unwanted radiation, provided that the applicator size is somewhat smaller than the prescribed tissue and that the aperture and anatomical curvatures are about the same.

The Bureau of Radiological Health is presently developing a safety performance standard for microwave diathermy equipment. An important goal is to minimize exposure of patients and operators to unwanted radiation. This can be achieved in two ways. One way is to use appropriate shielding enclosures around spaced applicators. It is feasible but can be somewhat cumbersome and, thus, not attractive from a clinical point of view. The other way is to use direct contact applicators for high power levels. These new types of applicators are not available for the microwave frequency of 2.45 GHz, although designs were developed for lower microwave frequencies in the 750 MHz to 1000 MHz range (1-5). The purpose of this paper is to examine the technical feasibility of using direct contact applicators for microwave diathermy operating at 2.45 GHz and to compare them with conventional types of applicators -- the spaced applicators. The emphasis is on temperature distributions as the measure of physical effect of microwave diathermy. An added goal of the direct contact investigation is to achieve uniform heating (excluding focusing of curved anatomy) instead of peaked heating, easily obtainable with unloaded or uniformly loaded rectangular waveguides. Clinical testing of these applicators has not been performed.

The main design features of the new and conventional types of applicators are described first, then the experimental methods to measure heating patterns in planar phantoms, leakage radiation and scattered fields are discussed. After presenting the experimental results, the heating patterns and the leakage measurements of direct contact applicators are compared with the heating patterns and the scattered fields of spaced applicators. In the conclusions, the major differences in performance of the new and conventional types of applicators are summarized.

MATERIALS AND METHODS

Applicators

New Types

In a previous study (6), both large and small direct contact applicators for 2.45 GHz were investigated. The large applicators are high power radiators (maximum of several hundred Watts at applicator input) and the small applicators are low power radiators (usually less than 10 Watts at applicator input). Only the large applicator results will be reviewed in this paper. They are prototypes of designs (or slight modifications of these designs) already commercially available for non-diathermy uses or new designs specifically intended for microwave diathermy. The above applicators were selected because of their different design characteristics. They still need to be tested clinically, and possibly modified to include a cooling mechanism to prevent excessive surface heating of treatment area, before they are made available to the users.

In the foreground of figure 1 are shown the four large applicators under investigation. A discussion of the main features of the selected large applicators according to increasing size follows.

a. Square aperture horn (second applicator from right). This horn (length of 8.3 cm) is circularly polarized with an input power carrying capability of 300 Watts and a frequency range of 2.4 GHz - 2.5 GHz. The basic design was not specifically developed for microwave diathermy; it is commercially available (Model No. 24 HC-3, Nurad, Baltimore).

b. Circular waveguide (see first applicator at left). This applicator is a standard circular waveguide (diameter 9.8 cm) in which the transverse TE_{11} mode propagates in the frequency range of 2.07 GHz to 2.83 GHz.

c. Teflon slab-loaded rectangular waveguide (second applicator from left). This is a standard WR 430 rectangular waveguide (width of 10.9 cm; height of 5.2 cm) partially loaded with two Teflon slabs 3.2 cm wide; one is on each side of the waveguide. A TEM mode is excited between the two dielectric slabs, thus providing for a uniform heating pattern in the center. (A patent application was filed. A paper describing the design performance of slab-loaded rectangular waveguides is in preparation for publication.)

d. Circular aperture horn (first applicator on right). This horn (diameter of 15.2 cm) is circularly polarized with an input power carrying capability of 300 Watts and frequency range of 2.4 GHz - 2.5 GHz. Its special design features consist of the output flange-choke (a choke assembly around the outside of the horn aperture) to suppress leakage radiation as well as a resistive termination at the back of the antenna to absorb reflections from a tissue load. It is commercially available (Model No. PN 82152-14C26700, Transco Products, Venice, CA).

The applicator in the background of figure 1 is a round aperture horn (diameter of 14 cm) that is commercially available (Model No. 24 HC-4, Nurad, Baltimore). It is not included in this study because its basic design is the same as that of the square aperture horn.

Conventional Types

Figure 2 shows typical high power spaced applicators presently in clinical use (7). Briefly, the Type A (left foreground) and Type B (right rear) are 10.2 cm and 15.2 cm hemispheres, respectively, while the Type C (right foreground) and Type E (left rear) are corner reflectors 12.7 cm by 11.4 cm and 17.2 cm by 14 cm, respectively. The Type B and Type E applicators are generally used at present in clinical practice.

Experimental Methods

Heating Patterns

The basic experimental procedure for measuring heating patterns induced in planar phantoms by the new applicators is the same as that for spaced applicators (8). A sketch of a typical experimental set-up is shown in figure 3. The planar phantom consists of simulated fat and muscle tissue prepared in accordance with the procedures developed by Guy (9). The fat thickness is 1 cm, the length is 60 cm, the width is 30 cm and the height is 10 cm. Briefly, a direct contact applicator, connected to a microwave diathermy machine by coaxial cable, is symmetrically positioned on top of the phantom with respect to the vertical midplane (top sketch). After microwave heating is completed, one half section of the phantom is quickly removed and the other half section is viewed by an infrared camera (bottom sketch) to obtain thermograms and temperature profiles. For the non-contact case, the conventional applicator is placed symmetrically above the phantom at a particular spacing.

Leakage Radiation

Leakage radiation for the direct contact case was measured by positioning an applicator on top of a planar phantom. A commercially available isotropic probe (Narda 8300) with its (5 cm) dielecric sphere scanned flush along the aperture-phantom boundary was used to measure maximum radiation.

Scatter Radiation

Since for spaced applicators radiation from the therapeutic beam can be scattered and subsequently impinge on operator or unprescribed tissue of patient, the term, scatter radiation, instead of leakage radiation used for the direct contact case, is more appropriate. Figure 4 shows a picture of the experimental set-up for scatter measurements of a loaded applicator (8). A Type B applicator is loaded with an 8 cm diameter diathermy arm phantom consisting of a 1 cm outer cylindrical layer of simulated fat material, filled with a muscle equivalent material.

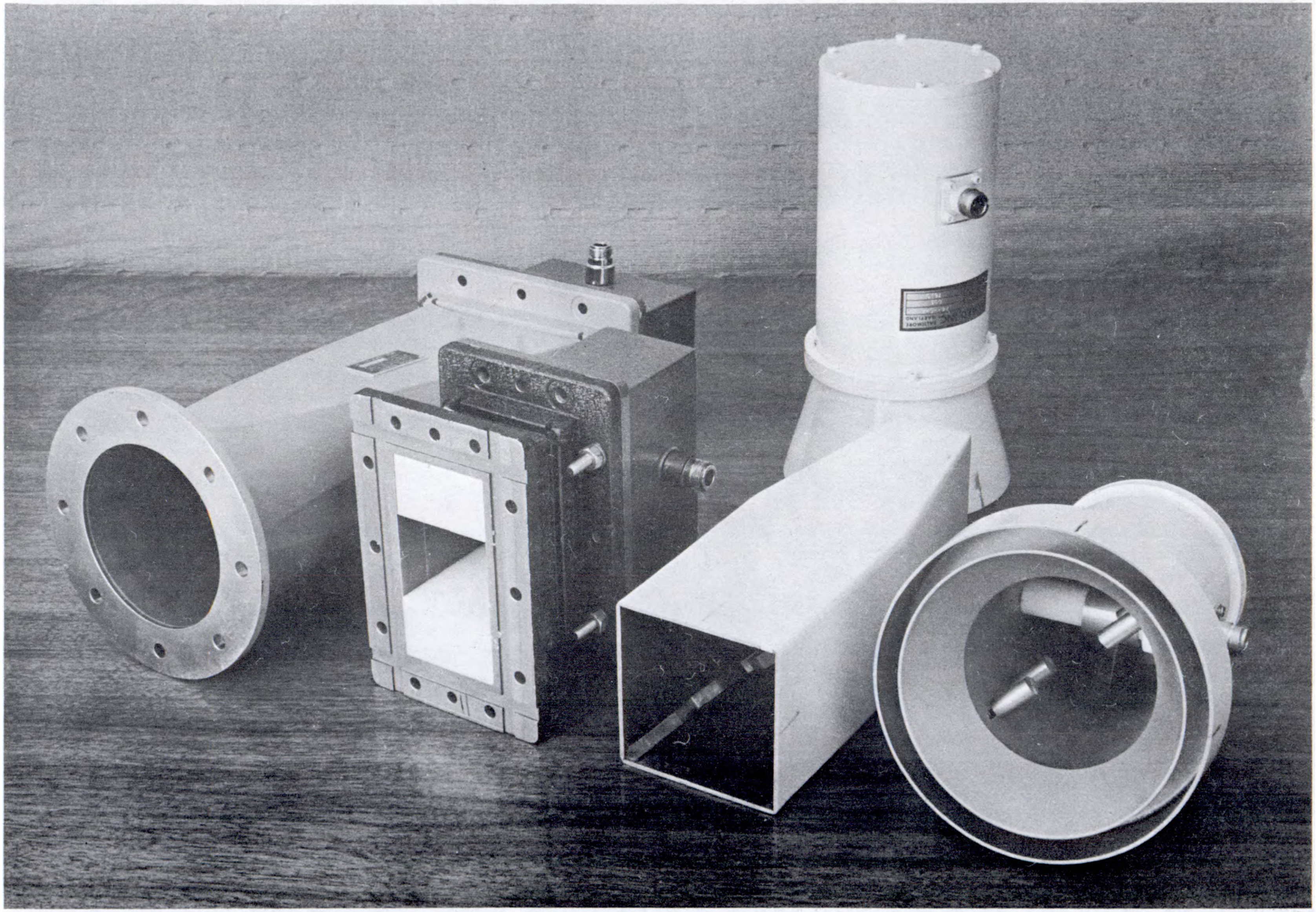
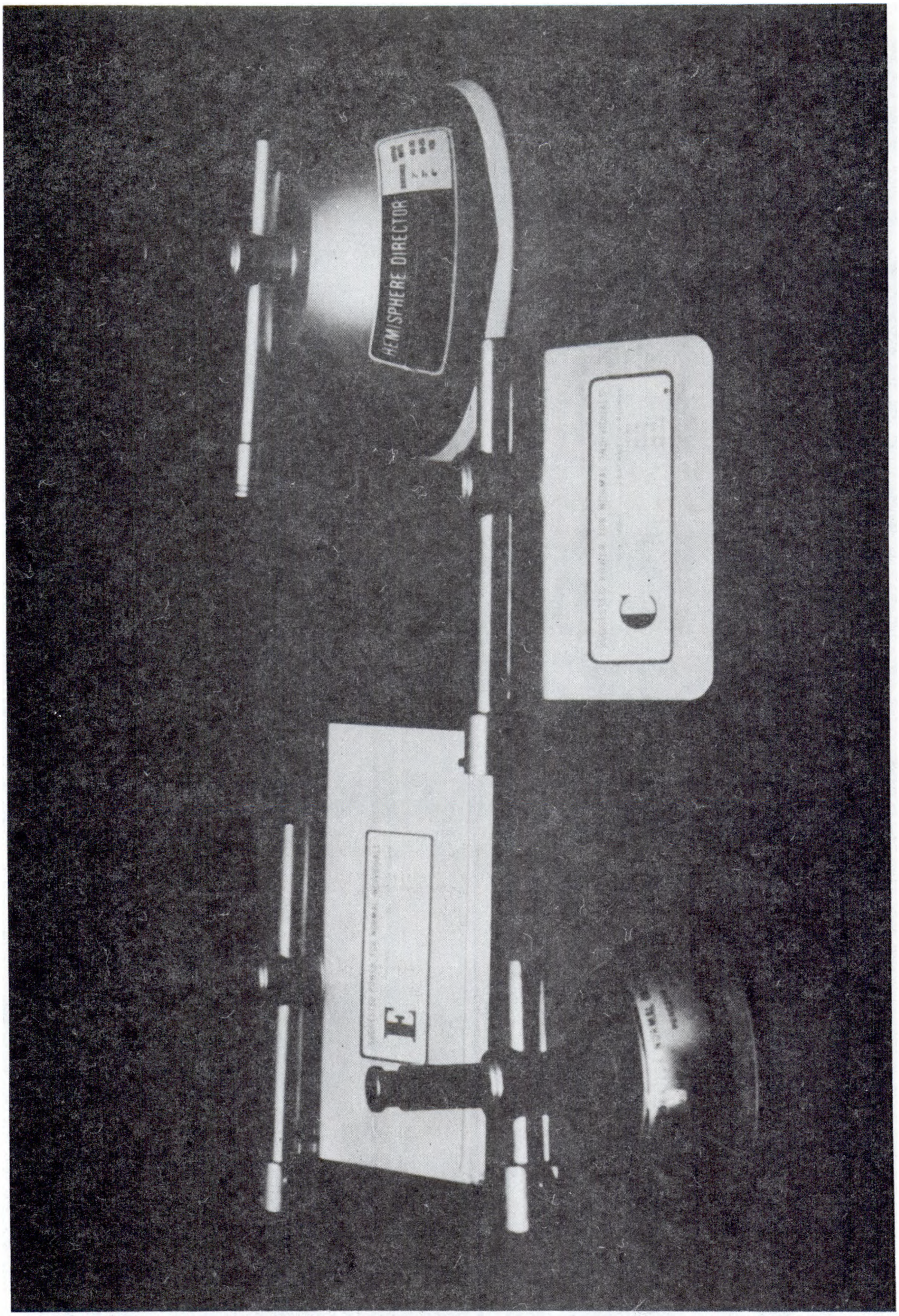
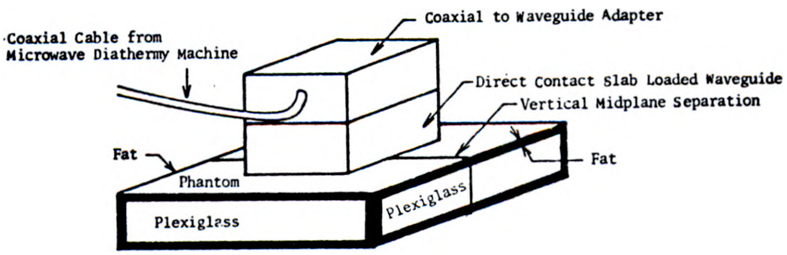


Figure 1

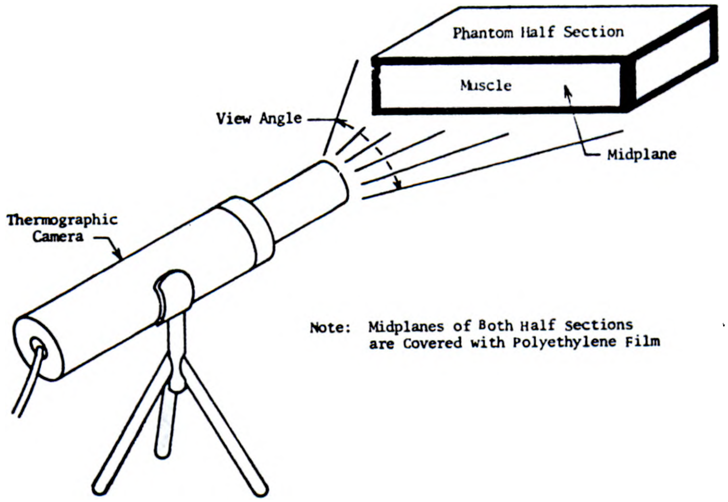


HEMISPHERE DIRECTOR
C
E

Figure 2


Coaxial to Waveguide Adapter
Coaxial Cable from Microwave Diathermy Machine
Direct Contact Slab Loaded Waveguide
Vertical Midplane Separation
Fat
Fat
Phantom
Plexiglass
Plexiglass

Microwave Heating


Phantom Half Section
Muscle
View Angle
Midplane
Thermographic Camera

Note: Midplanes of Both Half Sections are Covered with Polyethylene Film

Heating Pattern Measurement

Figure 3

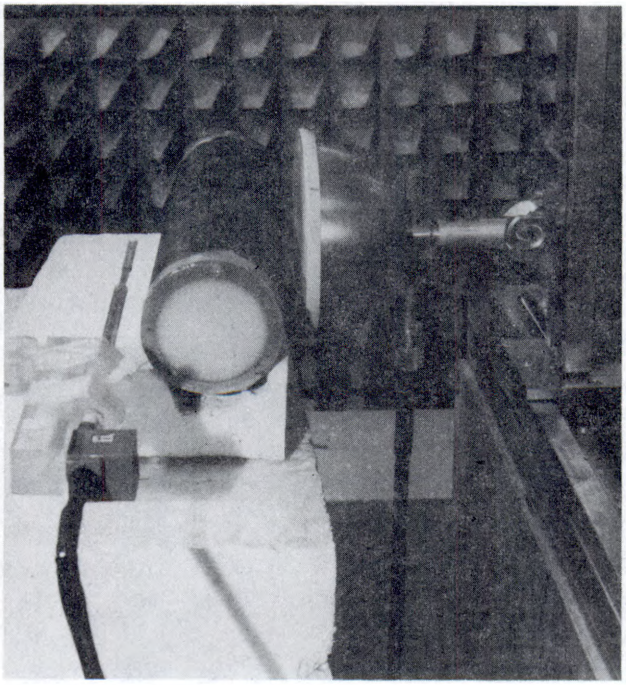


Figure 4

EXPERIMENTAL RESULTS

Heating Patterns

New Types of Applicators

The heating pattern results of the direct contact applicators are characterized in terms of two parameters, the heating pattern (width), w, and the heating pattern (depth), d (Fig. 5).

a. Width. The upper sketch in figure 5 shows a temperature profile similar to the one shown in figure 6. Such profiles are obtained by positioning the intensified white scan line in the region of maximum heating of the corresponding thermogram (see, for example, upper picture of figure 6). The temperature profile is labeled parallel to interface because the scan line in the thermogram of figure 6 is parallel to the fat-muscle interface of the planar phantom. The width, w, of this selected profile, as shown in sketch of figure 5 is defined as the width of the trace for which the temperature rise is half the maximum temperature rise ΔT. It was found convenient to position the cameras one meter from a phantom midplane so that measured lengths on the photographic trace must be doubled to obtain the real lengths (in terms of the graticule divisions, the horizontal scale factor is six divisions per 10 cm).

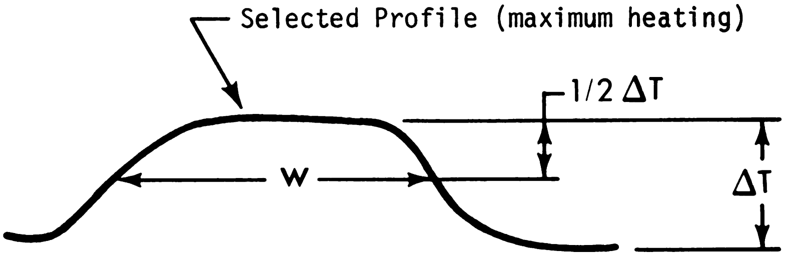
b. Depth. The definition of the penetration depth, d, is shown in the lower sketch of figure 5. First, the air-fat interface is located at the start of the steep temperature gradient (due to the heating of the fat). From this interface, the known fat thicknesses of a phantom is measured horizontally to obtain the fat-muscle interface and the profile temperature of (1/2)ΔT, shown in figure 5 (like for w, the profile lengths are half the real lengths). A profile normal to the interface like figure 7 is obtained for this comparative study by rotating a phantom ninety degrees counterclockwise from its initial position so that the air-phantom interface is at the left of trace (compare positions of phantom in thermograms of figures 6 and 7, respectively).

The phantoms were irradiated with a thermographic camera sensitivity of five corresponding to 1.25 degrees Celsius per two vertical divisions on temperature profiles. A power setting of 50 Watts was applied for 10 seconds. The resulting thermograms and temperature profiles of heating patterns in planar phantoms irradiated by typical direct contact applicators are shown in figures 6-9.

Figure 6 shows the heating pattern produced by the Teflon slab-loaded rectangular applicator. The pattern is uniform in the central region with w equal to 7.3 cm. The penetration depth d is determined from figure 7 and is equal to 2.2 cm.

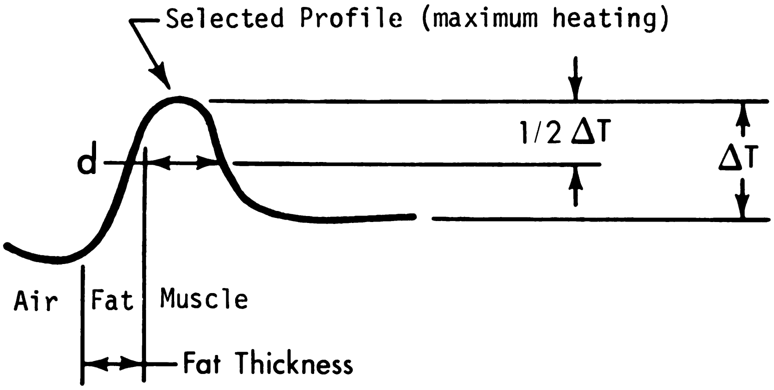
The two subsequent figures show both normal and parallel profiles superimposed on the same photo with the thermogram corresponding to the parallel profile. Figure 8 shows the heating patterns of the circularly polarized aperture horn with w equal to 7.5 cm and d equal to 2.2 cm. As in figure 6, the central portion of the parallel profile is uniform. Figure 9 shows the heating pattern of a circular waveguide with w equal to 5 cm and d equal to 2.2 cm. The parallel profile is less uniform than in the two previous cases, although the diameter is only 1.27 cm shorter than the length of the WR 430 applicator.

For the purpose of comparison of w and d, the new applicators listed in Table 1 are arranged according to increasing aperture size.



Heating Pattern Width of Profile Parallel to Interface

Selected Profile (maximum heating)
1/2 ΔT
w
ΔT

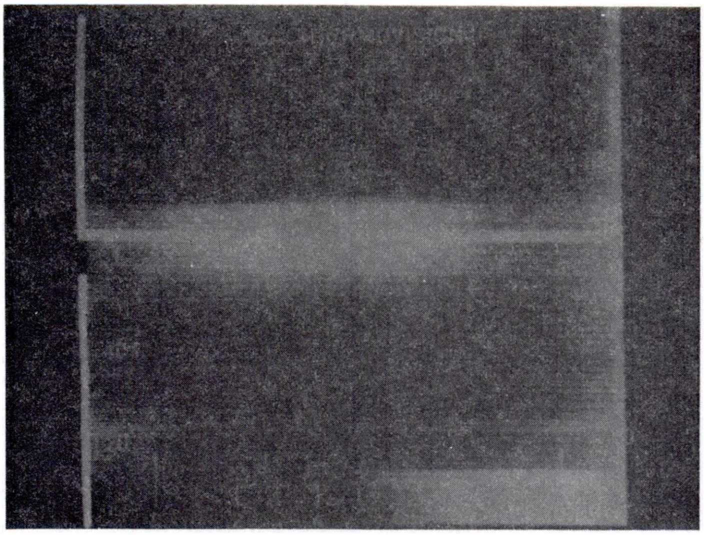


Heating Pattern Depth of Profile Normal to Interface

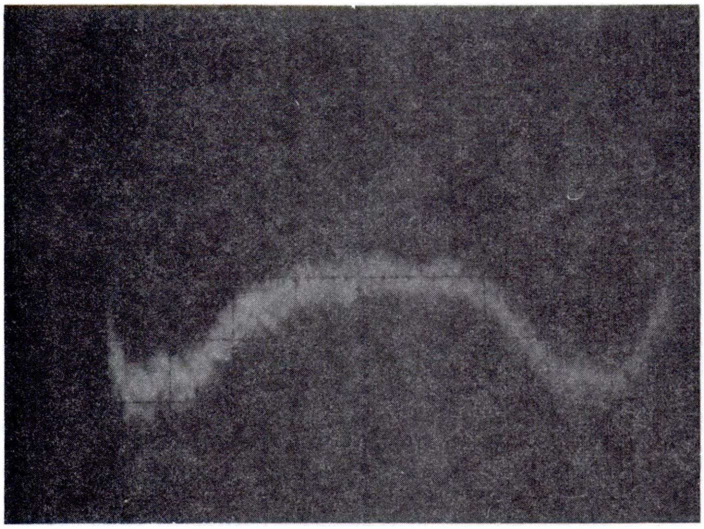
Selected Profile (maximum heating)
1/2 ΔT
d
ΔT
Air
Fat
Muscle
Fat Thickness

Figure 5

SLAB LOADED RECTANGULAR WAVEGUIDE



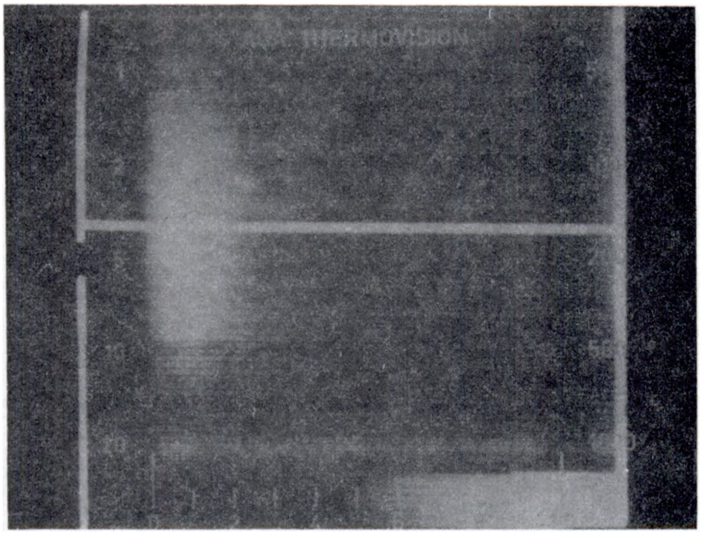
THERMOGRAM - PLANAR



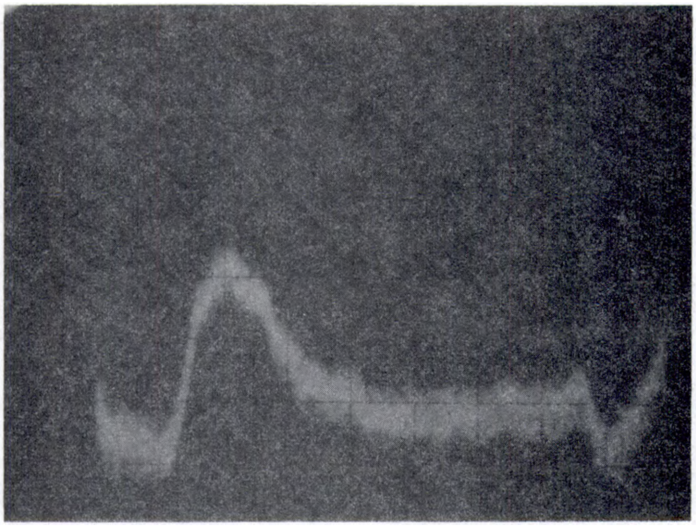
PROFILE PARALLEL TO INTERFACE

Figure 6

SLAB LOADED RECTANGULAR WAVEGUIDE


THERMOVISION

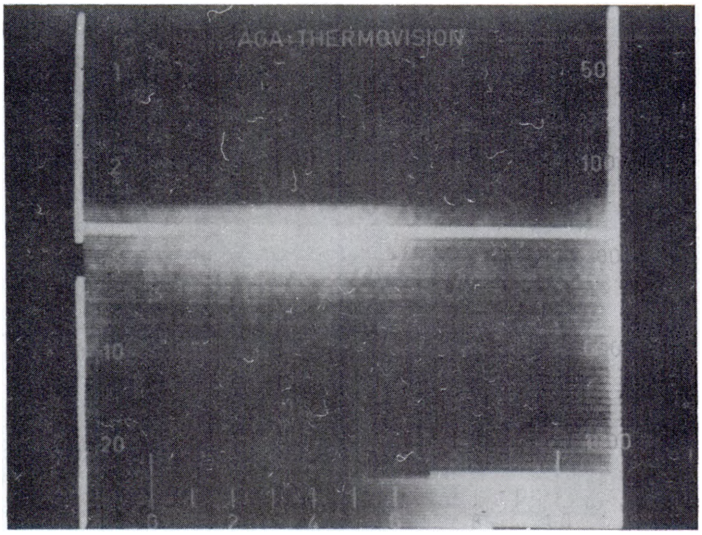
THERMOGRAM-PLANAR



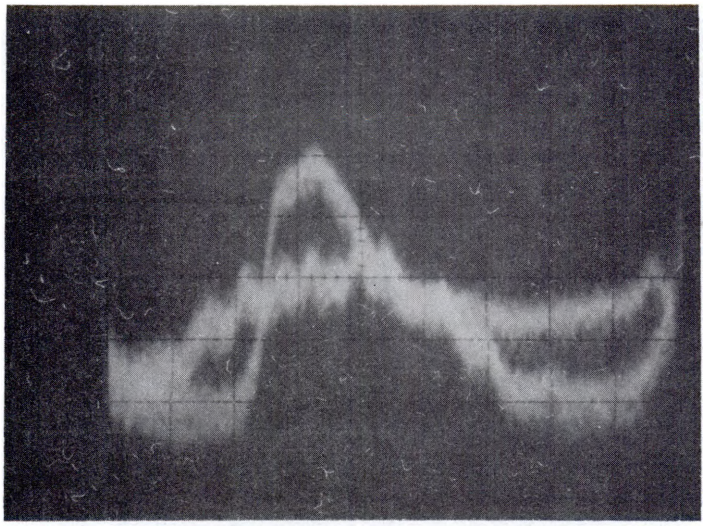
PROFILE NORMAL TO INTERFACE

Figure 7

CIRCULARLY POLARIZED ANTENNA


AGA-THERMOVISION
1
2
10
20
50
100
1000

THERMOGRAM - PLANAR



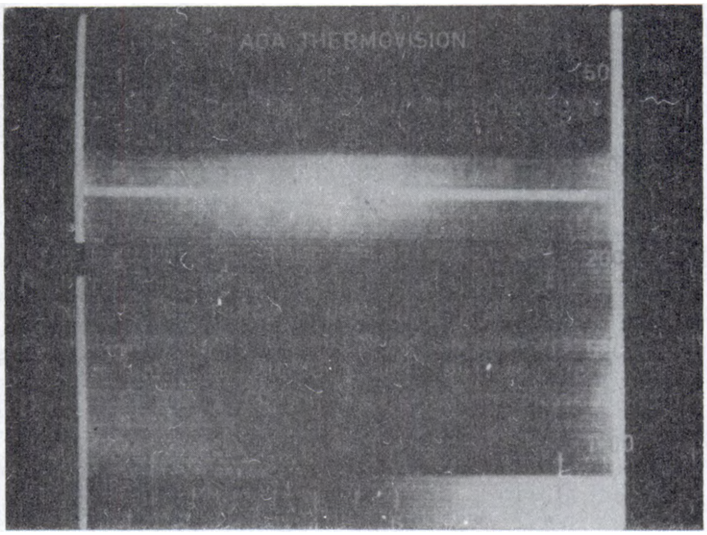
NORMAL

PARALLEL

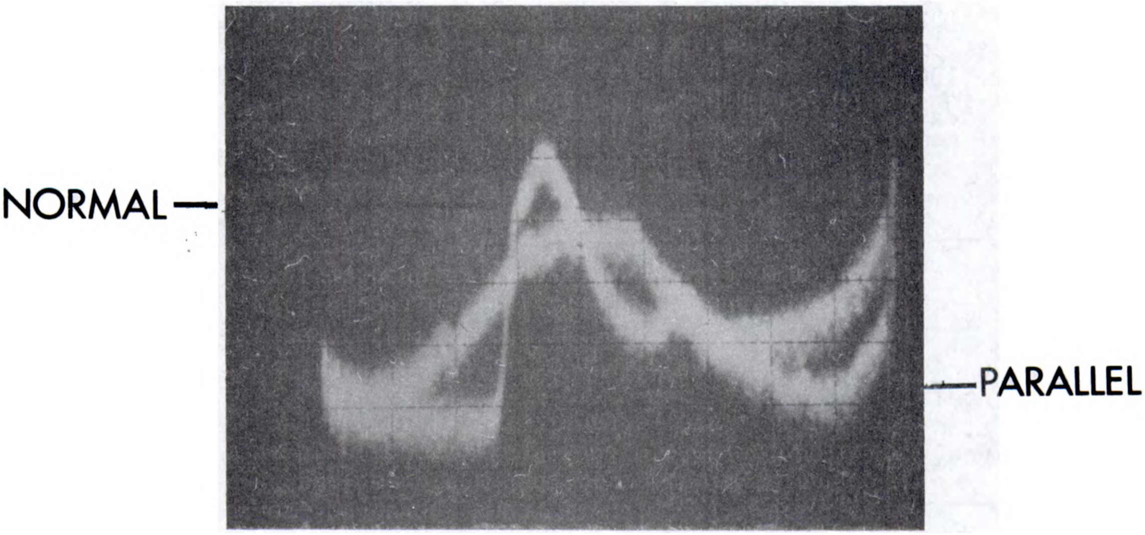
PROFILES NORMAL AND PARALLEL TO INTERFACE

Figure 8

CIRCULAR WAVEGUIDE


AGA THERMOVISION
50

THERMOGRAM - PLANAR



PROFILES NORMAL AND PARALLEL TO INTERFACE

Figure 9

Table 1. Typical heating patterns

Type of Applicator	Planar w (cm)	Phantom d (cm)
Square Applicator Horn	5.0	2.2
Circular Waveguide	5.0	2.2
Slab-Loaded Rectangular Waveguide	7.3	2.2
Circular Aperture Horn	7.5	2.2

Conventional Types

Figure 10 shows a thermogram and the corresponding temperature profile of a Type B applicator at a spacing of 5 cm between its aperture and the top surface of the irradiated planar phantom. This profile (refer to (8) for details) is clearly not uniform in the center. Instead, it is doughnut shaped with a marked minimum in the center. Figure 11 shows the heating pattern of the Typc C applicator for the same spacing. The temperature of this conventional applicator is uniform in the center.

Leakage Measurements

The leakage measurement results are shown in Table 2. The total uncertainty of these measurements is ± 2 dB, due to the calibration and ellipticity of the probe (see B in Materials and Methods Section). Planar phantoms were selected to obtain measurements under the most ideal conditions, since limb phantom loading of planar apertures could only increase leakage with less direct contact. The circular aperture horn with a flange-choke design (see Material and Methods) has negligible leakage, namely, 0.8 mW/cm^2 per 100 Watts. (The leakage of this horn is 4 mW/cm^2 per 100 Watts for a 1 cm dielectric shim between its aperture and the phantom.) The circular waveguide value is 2.3 mW/cm^2 per 100 Watts. The leakage of the loaded rectangular waveguide is 4.0 mW/cm^2 per 100 Watts. The square aperture horn has the highest leakage, namely, 11.6 mW/cm^2 per 100 Watts.

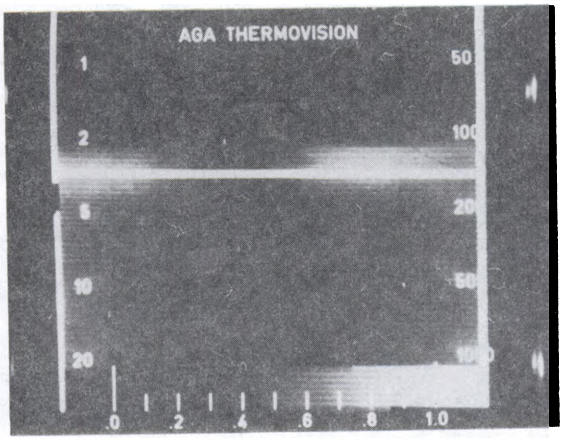
Table 2. Leakage measurements with planar phantoms

Type of Applicator	Maximum Leakage mW/cm^2 per 100 Watts
Square Aperture Horn	11.6
Circular Waveguide	2.3
Rectangular Waveguide (loaded)	4.0
Circular Aperture Horn	0.8

Scatter Measurements

Figure 12 shows a three-dimensional plot of the scattered field of a loaded Type B applicator (see reference (8) for details). Briefly, an 8 cm diameter arm phantom with 1 cm fat thickness is placed symmetrically in front of the applicator with a spacing of 2.5 cm between its aperture and the nearest point of the phantom's surface. In this figure, the x-y plane is at 2.5 cm behind the

TYPE "B" APPLICATOR


AGA THERMOVISION
1
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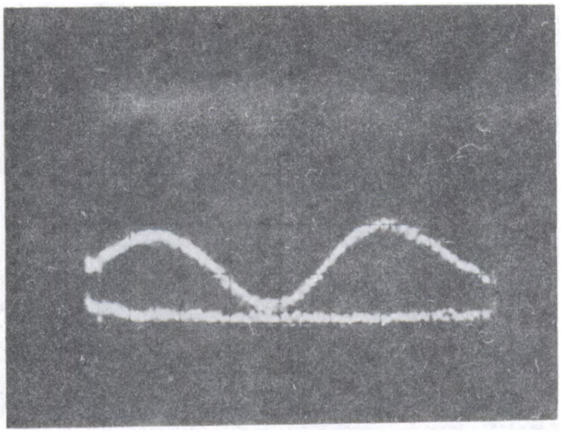
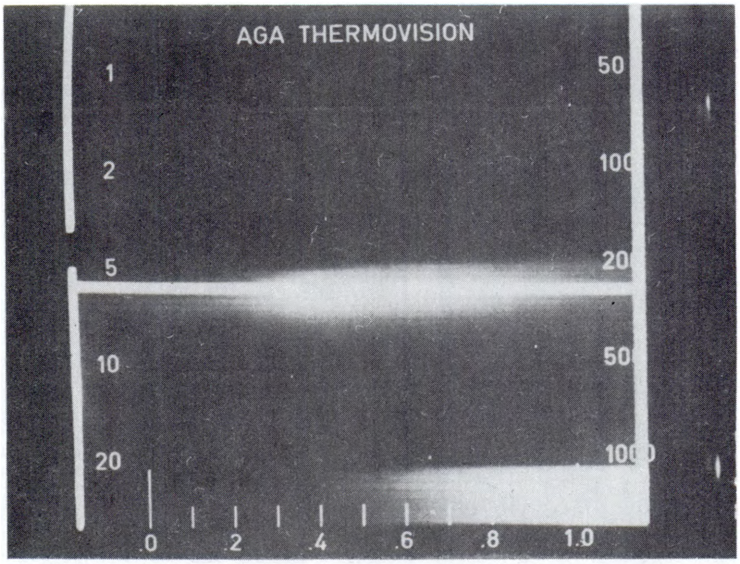
THERMOGRAM - PLANAR



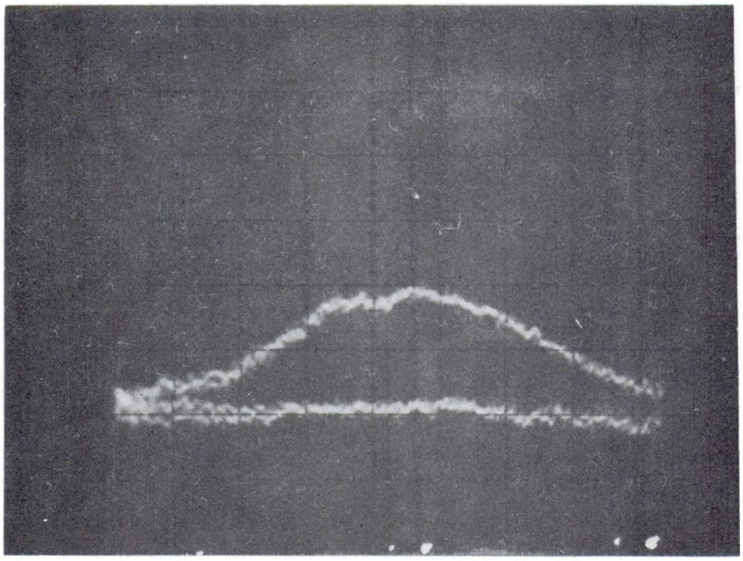
PROFILE PARALLEL TO INTERFACE

Figure 10

TYPE "C" APPLICATOR



THERMOGRAM - PLANAR



PROFILE PARALLEL TO INTERFACE

Figure 11

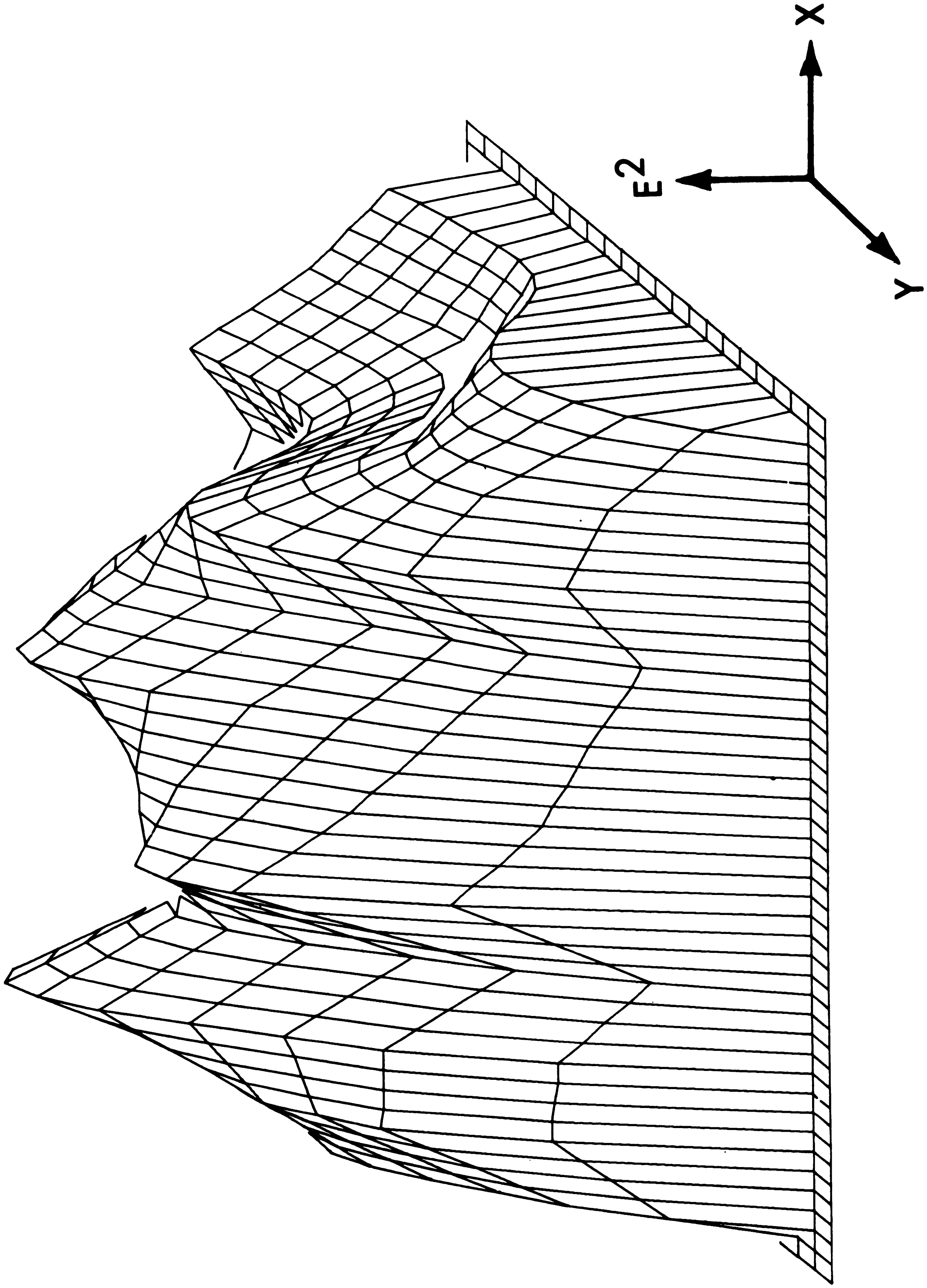


Figure 12

phantom (on the far side of the applicator) and parallel to it. The vertical scale is proportional to E^2, the square of the electric field. The electric field probe (10) is normalized to obtain an equivalent plane wave power density in mW/cm^2 in the near field ($E^2/120\pi$). The maximum of the scattered field is 30 mW/cm^2 per 100 Watts of forward power.

DISCUSSION

Heating Patterns

New Applicators

The heating pattern data shows that w, although increasing with aperture size, also depends on aperture field distribution. For example, a 1.27 cm increase in the width of the waveguide over the diameter of the circular waveguide results in an almost 50 percent increase in w. Table 1 shows that the d values are the same for the large applicators. This is expected because they are all loaded with a planar phantom so that no focusing of energy takes place. Also, data for these large applicators loaded with limb phantoms were obtained; however, the results are not included because they do not lend themselves to easy comparison. For large planar applicator-limb phantom combinations, leakage and scattered energy are significant due to reduced direct contact.

Conventional Applicators

It is well known (11) that the depth of penetration for microwave energy in planar tissue at 2.45 GHz is in the vicinity of 2 cm, as was demonstrated by the values of d in Table 1. Accordingly, only selected profiles parallel to interface are considered in this comparison of new and conventional microwave diathermy applicators. Both the Type A and Type B have symmetrical doughnut-shaped heating patterns resulting from their similarity in design. Although the Type E applicator, like the Type C applicator, is a corner reflector, its large size induces a somewhat doughnut-shaped heating pattern with a marked minimum off center and two peaks of unequal magnitude.

Leakage Measurements

The performance of the commercially available circular aperture horn applicator demonstrates that design techniques for minimizing unwanted radiation of direct contact applicators are available.

Scatter Measurements

The magnitude of the scattered and transmitted energy behind a phantom or patient strongly depends on the relative size of the applicator aperture, the phantom cross section, and the spacing between phantom and applicator. Thus, one must make scatter measurements for the specific applicator-phantom combination of interest to quantify the levels of fields which could expose other unprescribed tissue. There are two extremes which represent best and worst case hazard situations for unprescribed tissue or other persons in the vicinity. In one case, the phantom almost completely blocks the aperture so that the scattered energy is negligible. In the other case, the phantom minimally blocks the aperture so that essentially free space conditions exist. The data of figure 12 illustrates an intermediate case in spaced applicator loading. Three distinct peaks exist in the magnitude of $|E^2|$. The central (largest peak) is probably due to the effects of the large longitudinal field generated by the applicator, while the two lesser peaks may be due to the transverse field components.

CONCLUSIONS

The technical feasibility of using new types of applicators for microwave diathemy at 2.45 GHz, namely, direct contact applicators, is demonstrated. These new applicators still need to be tested clinically. The data presented in this paper shows that unwanted radiation that impinges on operator or unprescribed tissue of patient is much more easily controllable with direct contact applicators than it is with spaced applicators. In addition, more uniform heating patterns can be obtained with direct contact applicators than can be obtained with spaced applicators presently in general clinical use. One conventional applicator, the Type C applicator, has a heating pattern similar to the commercially available circularly polarized horn. However, this applicator is not any more in general clinical use.

The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health, Education, and Welfare.

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OPEN DISCUSSION

DR. OSEPCHUK: You mentioned a manufacturer of this circularly polarized applicator--who was that?

DR. KANTOR: Transco Products, and the person to contact would be John Glizer, if you have any questions. He is the director of engineering at Transco, Venice, California.

DR. OSEPCHUK: Secondly, have you ever made leakage measurements around any of these applicators with a person, rather than a phantom.

DR. KANTOR: Yes; I do not remember the figures now, but we had an applicator with a person, and we tried to evaluate the levels.

DR. OSEPCHUK: My basic question was: have you measured the difference in leakage close by with contact versus space applicators when applied not to phantoms but to various regular parts of the body?

DR. KANTOR: You mean with individuals?

DR. OSEPCHUK: Yes, people.

DR. KANTOR: You mean on a very systematic basis?

DR. OSEPCHUK: Or on any basis. The last part of this is, what is the maximum distance at which you find 10 mW/cm^2 in any case? In other words, even with the spaced applicators, how far away do you have to get before you read 10 mW/cm^2 when it is applied to a person anyplace?

DR. KANTOR: Perhaps some other people have done this; however, we were mapping the near field of the applicator which corresponds to the spacing that is stipulated by the manufacturer. If you recall, on these applicators it says, "Type B: use at a 2-inch spacing." So we said, "well let's see what is the field impinging at those levels."

DR. OSEPCHUK: What I am interested in is leakage, sprays.

DR. KANTOR: I call it a scatter.

DR. OSEPCHUK: No, what I am getting at is (this is my impression; I have not tested this thoroughly, but Bill Guy will be able to say something), that in most cases if you are three feet or more away, you are at 10 mW/cm^2 or below.

DR. KANTOR: At 3 feet away, it falls off very rapidly, that is true. However, we are, for example, concerned about not exposing tissue that is not supposed to be treated for one thing. We also are concerned about the physiotherapist when he tries to adjust the machine and so forth.

DR. OSEPCHUK: Another thing, if you are applying this contact applicator to the hand, how much radiation comes through the hand?

DR. KANTOR: We have not done this. But, of course, it is well known that for planar phantoms none of the radiation comes through.

DR. ROSENBAUM: How well matched are the applicators with regard to the coaxial input line?

DR. KANTOR: We plan to look at matching in the near future. Our emphasis was on matching patterns first since matching techniques at a particular microwave frequency are fairly routine. Transco tested their design under loaded conditions and measured a USWR of less than 2.

SESSION IV. GROWTH AND DEVELOPMENT

Moderator: *William M. Leach*

BIOLOGICAL EFFECTS OF MICROWAVES ON THE PUPAE OF TENEBRIO MOLITOR
Donald R. Green, Jr., Fred J. Rosenbaum, and William F. Pickard

NEUROENDOCRINE RESPONSES IN THE RAT AND DOG EXPOSED TO 2450 MHz (CW) MICROWAVES
Sol M. Michaelson, Ronnie Guillet, W. Gregory Lotz,
Shin-Tsu Lu, and Richard L. Magin

THE EFFECT OF REPETITIVE PRENATAL LOW-LEVEL MICROWAVE
EXPOSURE ON DEVELOPMENT IN THE RAT
Moris L. Shore, Richard P. Felten, and Anthony Lamanna

BIOLOGICAL EFFECTS OF MICROWAVES ON THE PUPAE OF TENEBRIO MOLITOR

Donald R. Green, Jr., Fred J. Rosenbaum and William F. Pickard
Department of Electrical Engineering
Washington University
St. Louis, Missouri

ABSTRACT

Current studies of microwave effects in T. **molitor** verify the occurrence of microwave induced teratogenesis at incident powers of 20 mW and above. Higher power experiments show a clearly thermal effect at power levels above 160 mW with 100 percent mortality at 320 mW. Finally, evidence is presented indicating that there is a difference in the susceptibility to microwave induced damage between healthy and weakened insects.

INTRODUCTION

Low power level microwave effects on the development of **Tenebrio molitor** pupae were first reported by Carpenter and Livstone in 1971 (1). Although exploratory in nature, and supported by relatively few experiments (140 irradiated insects), their findings prompted more extensive investigations in other laboratories (2-4). The principal finding of Carpenter and Livstone was that the irradiation of one to two day old pupae in a waveguide system at incident microwave power levels in the range 20-80 mW, at a frequency of 10 GHz, and for durations ranging from 20 to 120 minutes produced easily observable morphological defects in the emergent adults.

Their results were confirmed by Lindauer, et al. (2) who also investigated other experimental parameters such as pulsed versus continuous wave microwave power effects, field polarization, etc. The effect of dosage, defined as the power level-exposure duration product, was measured by Liu et al. (3) who reported a reciprocity relation. They found that if the dosage was kept constant, then nearly identical proportions of defective adults matured from pupae that had been irradiated for durations varying from 15 minutes to 480 minutes--a 1:32 time range. Tables 1 and 2 of reference (3) are reproduced here.

The defect classification scheme advanced by Carpenter and Livstone is used:

D insect died during pupation;
G1 insect developed head and thorax of an adult, but retained the abdomen of a pupa, sometimes with pupal case attached;
G2 adult insect had rumpled and grossly distorted elytra and/or shredded wings;
G3 adult insect was normal except for small discrete holes in elytra;
N adult insect was apparently normal.

Table 1. Incidence of teratogenic damage for 2 hours of exposure at various power levels

Group	D	G1	G2	G3	N	Total
20 mW	20 (26.7%)	11 (14.6%)	23 (30.7%)	7 (9.3%)	14 (18.7%)	75
10 mW	21 (26.7%)	10 (12.5%)	20 (25.0%)	5 (6.3%)	24 (30.0%)	80
2 mW	16 (23.9%)	6 (9.0%)	14 (20.9%)	2 (3.0%)	29 (43.3%)	67
1 mW	17 (23.3%)	6 (8.2%)	15 (20.5%)	2 (2.7%)	33 (45.2%)	73
0.2 mW	14 (20.9%)	5 (7.5%)	8 (11.9%)	1 (1.5%)	39 (58.2%)	67
0.1 mW	21 (20.0%)	7 (6.7%)	11 (10.5%)	1 (1.0%)	65 (61.9%)	105
0.05 mW	11 (16.2%)	4 (5.9%)	6 (8.8%)	0 (0.0%)	47 (69.1%)	68
Control	54 (18.1%)	13 (4.4%)	22 (7.4%)	1 (0.3%)	208 (69.8%)	298

Table 2. Incidence of teratological damage for dosage of 4 mWhr

Group	D	G1	G2	G3	N	Total
16 mW, 0.25 hr.	14 (23.7%)	6 (10.2%)	12 (20.3%)	2 (3.4%)	25 (42.4%)	59
8 mW, 0.5 hr.	14 (22.6%)	6 (9.6%)	13 (21.0%)	2 (3.2%)	27 (43.5%)	62
4 mW, 1 hr.	15 (24.6%)	5 (8.2%)	13 (21.3%)	2 (3.3%)	26 (42.6%)	61
2 mW, 2 hr.	17 (24.2%)	7 (10.0%)	14 (20.0%)	2 (2.9%)	30 (42.9%)	70
1 mW, 4 hr.	15 (23.8%)	6 (9.5%)	13 (20.6%)	2 (3.2%)	27 (42.9%)	63
0.5 mW, 8 hr.	15 (22.7%)	6 (9.1%)	13 (19.7%)	2 (3.0%)	30 (45.4%)	66
Control	28 (17.6%)	7 (4.4%)	12 (7.5%)	0 (0.0%)	112 (70.4%)	159

Two points concerning the results of reference (3) should be noted. First, the irradiation experiments were done at microwave power levels that produced an average temperature rise in the pupae that was measured as less than 2° C, using a thermocouple. In fact, at an incident power level of 20 mW, the measured temperature rise was 1.76° C. Second, no forms of damage were observed in adults that had been irradiated as pupae, that were not also found in untreated Tenebrio (5-7).

Recent work by the authors has been directed to more completely delimit the effects of low level microwaves on Tenebrio. Among our goals are: (i) to investigate the cumulative effects of microwave dosage as a function of pupal age at irradiation, (ii) to determine relative susceptibility to microwave induced damage during different stages of metamorphosis, (iii) to investigate the effect of the frequency at which the microwave power is applied, and, (iv) to demonstrate that the microwave power levels with which significant increases in teratogenesis have been associated are far below the power level at which obvious thermally induced damage occurs. In this paper we report the current status of our investigation.

MATERIALS AND METHODS

Larvae are obtained comercially from Rainbow Mealworms, Compton, California. Their "large" grade larvae are normally shipped on newsprint. Several experiments were performed with samples shipped in grain but no significant differences were noted. The larvae are kept at room temperature in porcelain steel 8" diameter pans, with approximately 500 to 1000 larvae per pan; pans are stored in darkened, room temperature cabinets. Larvae are fed on Special K™ breakfast cereal, with the cereal covering the larvae. Wafers of potato are added every day to provide needed moisture. Newly shipped larvae usually begin to pupate within one week of arrival.

The pans are culled each morning and new pupae are removed using a porcelain spoon. Each day's yield is microscopically inspected and segregated into three groups: "ideal," "nonideal," and "discards." The "ideal" pupae are well formed, with symetrically folded limbs and wings, free from obvious defects, marks, or any evidence of prior trauma. Approximately 30 percent of the pupae are ideal. Nonideal pupae may have missing, distorted or improperly folded limbs. Pupae with black spot lesions or pigmentation abnormalities are also placed in the nonideal category. Nearly 60 percent of the pupae are nonideal. Pupae that appear damaged or defective to an extent that precludes probable survival during metamorphosis are discarded. These represent about 10 percent of the total yield.

Experiments are performed using either exclusively ideal or nonideal pupae. Pupae are microscopically examined and their sexes are determined. After selection for an experiment, the pupae are randomly separated into control and irradiation groups. Both groups receive simultaneous and identical handling--except for the application of microwave radiation. All pupae are placed in styrofoam holders that are then inserted in an X-band (WR-90) rectangular waveguide. See reference (2) for details.

Upon completion of the irradiation, both irradiated and control insects are placed in individual, numbered, disposable ml polystyrene beakers (approximately 1" diameter x 1" high). The insects are then kept in a darkened environmental chamber at 21° C for the duration of metamorphosis, which is typically 9 days to 2 weeks. The relative humidity during experiments performed in the winter ranges from 15 percent to 25 percent which is below the optimum ambient humidity for the development of Tenebrio (7).

The adults are visually inspected for defects within 24 hours after they emerge from their pupal cases, and are classified according to the scheme of Carpenter and Livstone (1). We have recently modified this classification scheme to record finer gradations in the type and extent of damage. In some of our experiments, the insects are reclassified one week after initial classification to see whether significant changes developed as the adult matured. No significant changes have so far been observed.

RESULTS

The current series of experiments were begun in June 1976. Our first interest was to establish baseline data with new **Tenebrio** cultures for comparison with the damage distribution obtained in our laboratory about 2 years earlier. Since June 1976 approximately 1500 pupae have been irradiated with an equal number simultaneously employed as control specimens.

Initial results showed significant departures from the previous data. As many as 80 to 90 percent of the subjects in both irradiated and control categories either did not survive metamorphosis, or emerged as deformed adults. Cultures obtained from a second supplier exhibited similar results. A consistently large proportion of pupae harvested during this period were afflicted with external, black scablike adhesions with an underlying whitish pulp. "Black spots" were also noted on some larva.

Samples taken from the lesions of representative specimens were cultured and the presence of fungi from the yeast groups **Geotrichum candidum** and **Trichosporon** were discovered. New hygenic procedures were initiated and the percentage of damaged or dead emergent adults in the control group was reduced to about 40 percent compared to approximately 30 percent as previously observed (2,3). We now believe that cannibalism by active young larvae on passive prepupescent larvae or first day pupae produces host sites for the stimulated growth of the fungi which are otherwise part of the normal flora of **T. molitor**.

Similar lesions were noted by Steinhaus and Zeikus (8) who described them as being "large, white, and containing melanized material." They go on to state that "this type of larva has difficulty in moulting, and is always noted to emerge as a grotesque pupa with huge blisters in the wing or wing covers."

Table 3 shows a comparison of the characteristics of the control groups in a number of previously reported experiments. All types of damage are summed and displayed as G. Group ε represents the characteristics of the "ideal" pupae while group ξ is for the "nonideal" category. Group δ is an experiment done during the recovery following the period of diseased cultures in the summer. We note that 60 percent to 70 percent of the controls mature normally. Currently 20 percent to 30 percent are defective and less than 10 percent die. This compares with nearly twice as many dead and half as many damaged in previously reported work (groups β and δ).

We believe that 70 percent normals represent a realistic measure for the controls. The percent abundance between the dead and damaged control insects, however, seems to have drifted substantially from the unsorted controls of experiments performed in 1973 and 1974.

Notice that the nonideal pupae (group ξ) control show fewer normals, and more damaged and dead insects than any other group except group δ. Results during the recovery period tended to drift but have now stabilized in the vicinity of the group ε results for ideal pupae and the group ξ results for the nonideals.

Table 4 lists the characteristics of the experiments done in the current series. The results of the completed experiments are presented in Table 5. The results are presented as numbers of insects in the various categories and the probability, p, that the observed differences between irradiated and control groups were obtained by chance from a homogeneous population (9). Also included is the ratio of the insects in the category to the total number expressed in percent.

DISCUSSION

The results shown in Table 5 clearly indicate that there are significant differences between the control categories and the irradiated insects. The probabilities that this difference could occur by chance range from 0.3 in some experiments to less than 0.006. Thus we believe that low-level microwave effects in T. **molitor** are once again confirmed; however, not at the same absolute levels as previously reported (2,3), as can be seen by comparison with Tables 1 and 2.

Reference to the control characteristic presented in Table 3 show that there is variability in various categories as a function of time and between groups as a function of the selection procedures. Experiments done with samples chosen at random from a "wild population," an unsorted mix of ideal and nonideal pupae, should lead to absolute results between the "ideal" (9000 series) and the nonideal ones (M series). Earlier experiments (2,3) were done using a wild population which may have included some specimens currently classified as "discard" and so, considering the drift in the control categories, detailed comparisons between previous work and current work present certain difficulties.

Comparison between the (9000 series) ideal case and the nonideal (M series) results suggests that there may be substantive differences in the susceptibility of previously damaged insects to microwave induced damage. This concept is at present being examined by means of a set of experiments identical to the ABCD series, but using only nonideal pupae.

The possibility that the condition of the pupa affects its susceptibility presents a hypothesis for the observation that in earlier work (3) there were clearly two classes of insects: those which were readily affected by microwaves and those which were more resistant. If the logarithm of the percent number of normal insects from the results in Table 1 is plotted against the incident power then the curve shown in figure 1 is obtained. Notice that percent normals decrease very rapidly with only very small amounts of incident power. This suggests a highly susceptible group is also present as represented by the much more gradual reduction in the percent normals at the higher power levels. In fact, the incremental susceptibility of both groups seems constant.

CONCLUSIONS

Current studies of microwave effects in **Tenebrio molitor** continue to verify the occurrence of microwave induced teratogenesis at the 20 mW power level and above. At 320 mW for 2 hours at 9 GHz there is 100 percent mortality, while at 160 mW for two hours, there is only 10 percent mortality--indicating that the onset of lethal (presumably) thermal effects occurs within this power interval. An average temperature rise of less than 2° C at 20 mW of incident power at 9 GHz is expected from previous thermocouple measurements (2). Therefore, at the 320 mW power level a temperature rise above ambient of about 30° C is expected--high enough for macromolecular heat denaturation. Thus, effects observed at and

Table 3. *T-Molitor* control group data

INVESTIGATORS	% RATE OF OCCURENCE		
	Normal	Defective	Died During Metamorphosis
α - Carpenter & Livstone(1)	89.0%	5.9%	5.1%
β - Lindauer et.al.(2)	64.0	15.5	20.5
γ - Liu et.al.(3)	70.0	12.0	18.0
δ - Green (summer)	59.0	40.0	1.0
ε - Green (current) ideal	71.2	28.3	1.7
ξ - Green (current) nonideal	51.3	34.9	9.6

Table 4. List of current experiments

SERIES	DESCRIPTION (All pupae one day old)	PURPOSE
9000	20mW for 2 hrs. Ideal Population	Establish baseline results.
B	40mW for 2 hrs. Ideal Population	Power varied in search of thermal effects.
A	80mW for 2 hrs. Ideal Population	"
C	160mW for 2 yrs. Ideal Population	"
D	32mW for 2 hrs. Ideal Pupae	"
M	20mW for 2 hrs. Non-ideal pupae	Compare susceptibility of ideal and non-ideal pupae.
L	20mW for 16 hrs. Ideal pupae	Extend continuous cumulation results.
S	100mW for 2 hrs. Non-ideal pupae	Examine susceptibility of non-ideal pupae.

Table 5. Summary of experimental results

Protocol: One day old "ideal" pupae - 9 GHz / 40 mW / 2 hr.
Series: B

	N	G	Misc.	Totals
Irradiated	66 (55.9%)	49 (41.5%)	3 (2.5%)	118
Control	84 (71.2%)	30 (25.4%)	4 (3.4%)	118
Totals	150	79	7	236

$X^2 = 6.872$ $p = 3.22\%$

Protocol: One day old "ideal" pupae - 9 GHz / 80 mW / 2 hr.
Series: A

	N	G	Misc.	Totals
Irradiated	77 (64.2%)	39 (32.5%)	4 (3.3%)	120
Control	71 (59.2%)	48 (40.0%)	1 (0.83%)	120
Totals	148	87	5	240

$X^2 = 3.837$ $p = 14.7\%$

Protocol: One day old "ideal" pupae - 9 GHz / 160 mW / 2 hr.
Series: C

	N	G	Misc.	Totals
Irradiated	54 (47.4%)	48 (42.1%)	12 (10.5%)	114
Control	76 (66.1%)	36 (31.3%)	3 (2.6%)	115
Totals	130	84	15	229

$X^2 = 10.85$ $p = 0.44\%$

Protocol: One day old "ideal" pupae - 9 GHz / 320 mW / 2 hr.
Series: D

	N	G	Misc.	Totals
Irradiated	0 (0%)	0 (0%)	16 (100%)	16
Control	10 (62.5%)	5 (31.2%)	1 (6.3%)	16
Totals	10	5	17	32

$X^2 = 28.2$ $p = 7.4 \times 10^{-5}\%$

Protocol: One day old "ideal" pupae - 9 GHz / 20 mW / 2 hr.
Series: 9000

	N	G	Misc.	Totals
Irradiated	37 (47.4%)	39 (50.0%)	2 (2.6%)	78
Control	47 (59.5%)	31 (39.2%)	1 (1.3%)	79
Totals	84	70	3	157

$X^2 = 2.408$ $p = 30\%$

Protocol: One day old "nonideal" pupae - 9 GHz / 100 mW / 2 hr.
Series: S

	N	G	Misc.	Totals
Irradiated	15 (25.4%)	35 (59.3%)	9 (15.3%)	59
Control	30 (50%)	20 (33.3%)	10 (16.7%)	60
Totals	45	55	19	119

$X^2 = 9.210$ $p = 1.0\%$

Protocol: One day old "ideal" pupae - 9 GHz / 20 mW / 16 hr.
Series: L

	N	G	Misc.	Totals
Irradiated	54 (45%)	60 (50%)	6 (5%)	120
Control	71 (60%)	36 (30%)	12 (10%)	119
Totals	125	96	18	239

$X^2 = 10.30$ $p = 0.58\%$

Protocol: One day old "nonideal" pupae - 9 GHz / 20 mW / 2 hr.
Series: M

	N	G	Misc.	Totals
Irradiated	38 (32.8%)	57 (49.1%)	21 (18.1%)	116
Control	59 (51.3%)	40 (34.8%)	16 (13.9%)	115
Totals	97	97	37	231

$X^2 = 8.15$ $p = 1.7\%$

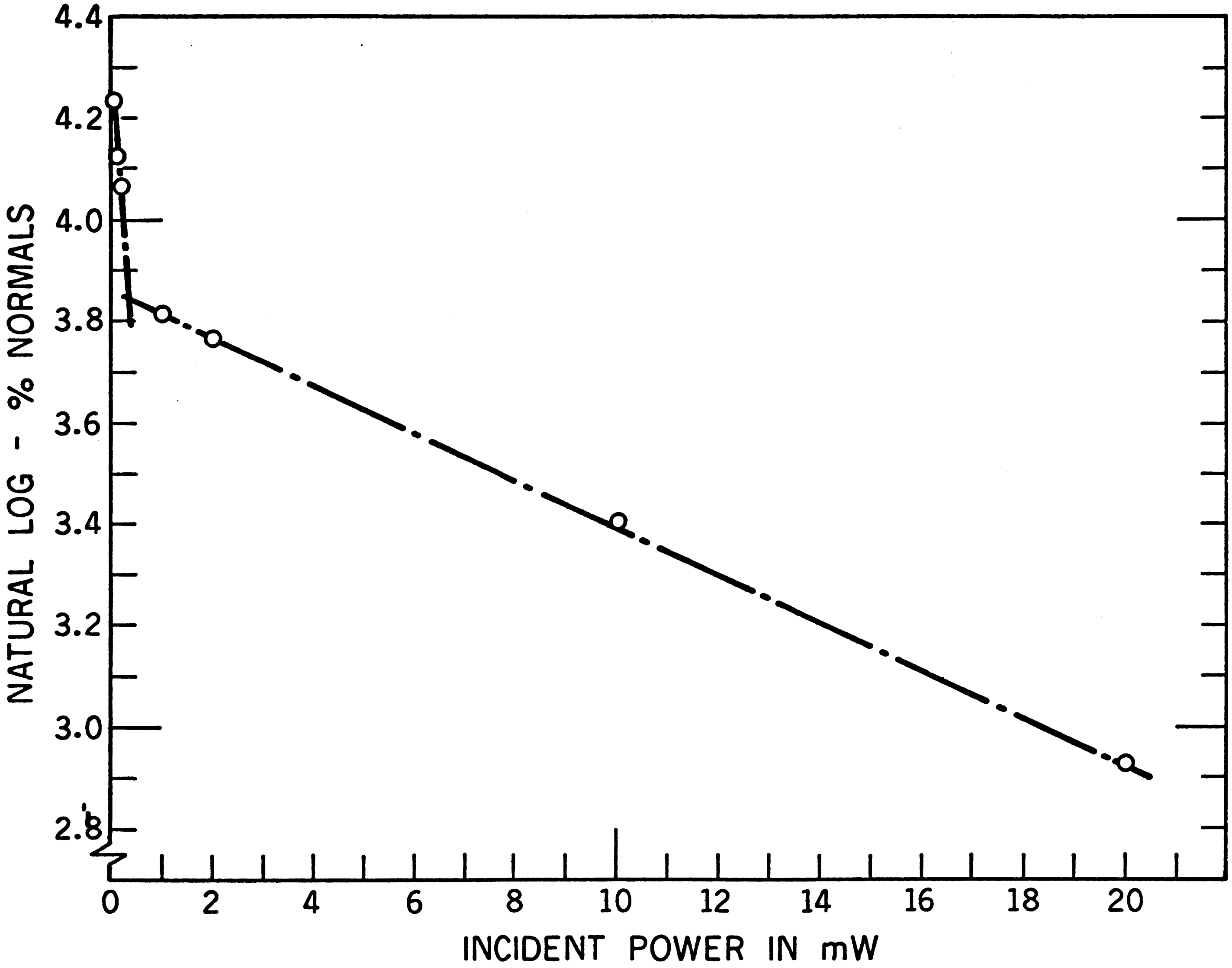

Figure 1. Normal population of irradiated insects as a function of incident microwave power level for 2-hour exposures at 9 GHz.

below the 20 mW power level are probably not thermal in the usual sense of the word.

Finally, there appear to be two classes of microwave susceptible insects indicated in these studies. Initial results suggest that previously traumatized pupae will be more likely to suffer damage from microwave exposure than those that show no signs of abnormality. Whether enhanced microwave susceptibility results from prior stress or trauma--or whether microwave susceptible individuals are also more susceptible to other trauma has yet to be investigated.

ACKNOWLEDGMENT

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OPEN DISCUSSION

DR. CARPENTER: I am very pleased that this work is being carried on at St. Louis. I think it is a rather ideal form when you do not have to worry about the thermal effect because the animal is whatever his environment is until he gets too hot to survive, which has been found to be 42°. Meal worms as a rule, are not around places where the temperature gets to be 42° in the summer; if they are, they run for the shade. It, therefore, is an ideal form to study without some extraneous factors. The fact that we had such good survival in our early experiments may be because they were performed in a new building, and we did not have any fungi around. However, I think that the most important thing is the possibility of two strains. I hope you go on some more with this.

DR. JUSTESEN: Have you run a cooling curve on any of these yet?

DR. ROSENBAUM: We did on the earlier results, and I did not bother to show it. The samples cool to ambient in about 8 minutes.

DR. BUFFLER: Was this a blind experiment? When you were selecting after irradiation, did you know from which group the animals had come?

DR. ROSENBAUM: This was actually double blind. As it is turning out; it seems to be totally blind.

NEUROENDOCRINE RESPONSES IN THE RAT AND DOG EXPOSED TO 2450 MHz (CW) MICROWAVES

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ABSTRACT

Changes in endocrine function and hormone levels occur in rats and dogs acutely exposed to certain power-density/time durations of 2450 MHz (CW) microwaves. Such effects are, however, of a transient nature and are related to temperature increase of the individual gland or hypothalamic-hypophyseal responsiveness as a result of increased body temperature. Power density of 20-30 mW/cm^2 seems to be a transitional range for stimulation of pituitary-adrenal activation in the rat.

INTRODUCTION

Of the many coordinating mechanisms in the body, the endocrine and nervous systems are of greatest functional importance. Although endocrine function is slower than neural, the two systems interact with and influence each other, thus constituting the neuroendocrine system.

Changes in the functional aspects of the neuroendocrine system as a result of microwave exposure have been reported and are considered to be caused by stimulation of hypothalamic-hypophyseal activity, resulting in changes in production, excretion, or utilization of trophic hormones. It has been hypothesized that this stimulation may be a result of a direct effect on the central nervous system, a direct or indirect thermal effect on a particular endocrine gland or an indirect stress effect, e.g. a thermal stress on the body producing a hypothalamic pituitary response.

In 1967 we reported that exposure of the dog to 2880 MHz pulsed microwaves at power densities above 100 mW/cm^2 resulted in physiologic responses (1) indicative of adrenocortical stimulation that was consonant with the concept of nonspecific "stress" (2). We suggested that microwaves of high-power density can act as a "stressor" affecting regulatory and integrative homeokinetic activity, resulting in an alteration in homeostasis. The proposed mechanism was accomplished by stimulation of the hypothalamic-hypophyseal adrenal axis. More recently, Dumansky and Shandala (3) reported adrenocortical changes in animals chronically exposed to "low-power density" microwaves, below 1 mW/cm^2. This takes on added significance in view of the evidence of adaptation to repeated microwave exposure as described by several investigators (1,4,5,6,7,8). In this context, it should be pointed out that activation of adaptive reactions could lead to maintenance of body function at some altered level of effectiveness or to the classical sequence of events--stress, adaptation, and exhaustion (9). These findings suggest that microwave energy absorption at certain power-density/time relationships can influence physiologic integration. The integrative mechanisms appear to be organized to respond selectively in patterns that may be quite different according to the specific stimulus.

In other studies we showed that in the young rat (8-12 weeks of age) exposed to microwaves, growth hormone response is altered at various power densities (10). At 10 mW/cm^2 there is an increase in growth hormone; whereas, at 40 mW/cm^2 rat serum growth hormone drops, after 60 minutes of exposure, to significantly low levels. The results of these studies support the concept that exposure of rats to high (thermogenic) levels of microwaves for periods of 1 hour or longer represents hypothalamic-hypophyseal activation, no doubt as a result of the thermal burden to the animal.

To obtain a better picture of neuroendocrine responses to microwave exposure, we have attempted to measure several hormones, simultaneously, in relation to different power-density/time combinations of whole-body and regional microwave irradiation. Such studies should provide a broad view of hormonal response in terms of patterns or profiles of overall balance relative to physiologic costs to the animal to maintain homeostasis while thermo-regulating to minimize the effects of microwave energy absorption.

MATERIALS AND METHODS

The studies to be described were performed on dogs subjected to head or thyroid irradiation and rats exposed whole-body in the far-field in an anechoic chamber.

A Raytheon CMD-10 or CMD-5 medical diathermy unit that generates 120 Hz modulated 2450 MHz microwave energy with output power of up to 125 watts was used for the regional exposure of dogs. The dogs were mongrels that had been acclimatized to the laboratory environment. Head irradiation was accomplished by exposing the head only to microwaves with the rest of the body shielded with microwave-absorbing material. The nuchal arch of the dog was used as a landmark and was placed 18 cm directly below the posterior margin of a rectangular horn. A Narda Isotropic Microwave Radiation Monitor model 8305 was used to measure the electromagnetic field. Microwave exposure was performed on animals under pentobarbital anesthesia. The details are described by Lu, et al. (11) and Kinnen et al. (12).

For localized thyroid irradiation an applicator was fabricated from C-band waveguide to coax adapter and loaded with a low loss dielectric as described by Magin, et al. (13,14,15). The applicator was applied directly to one thyroid gland of pentobarbital anesthetized dogs.

For whole-body (unanesthetized) rat exposures, the microwave power generators were a Raytheon PGM-100 and a Holaday 1200, providing 2450 MHz CW power. The animals were irradiated dorsally in the far-field zone ($\sim$160 cm) of an S-band standard gain horn antenna. Power density measurements were made with a Narda Model 8315 radiation monitor in the absence of the rats and exposure cages.

Long-Evans hooded male rats (Blue Spruce Farms, Altamont, NY) 250-350 grams, were used in these experiments. The animals were maintained in a constant temperature (24 ± 1°C) and a 12-hour (0600-1800) light/dark cycle with food and water ad libitum.

In the rat studies, the animals were "gentled" for 2 weeks prior to exposure by handling and at least three periods of conditioning in the exposure cages. During exposure, the rats were individually housed in nonrestraining FR

100 styrofoam cages, separated by 3-4 inches from other cages to reduce animal-to-animal field perturbations. The average power density for each cage area was the same (± 5 percent) with the power density distribution pattern for that area being less than ± 1 dB from the average. The details of these exposure conditions are described by Lotz and Michaelson (16,17) and Guillet, et al. (18).

On the day of the experiment, the animals were placed in the exposure chamber at 8 a.m., and the exposure was started after a 3-hour equilibration period. The 3-hour equilibration period was necessary (10) to assure that the rats' temperatures and corticosterone had returned to normal baseline levels at the beginning of exposure. All exposures were carried out between 11 a.m. and 2 p.m. to minimize the influence of circadian rhythm in temperature and circulating hormone levels. Two control (nonirradiated) rats were subjected to the same procedure, except for irradiation, as the four exposed rats in each experiment.

Exposures of 30 and 60 minutes were carried out at average incident power densitites of 13, 20, 30, 40, 50 and 60 mW/cm^2, or for 120 minutes at 13, 20, 30 or 40 mW/cm^2. At the end of the exposure, animals were decapitated and colonic temperature was taken immediately. Blood was collected in iced tubes containing EDTA; the plasma was assayed for corticosterone by a competitive protein-binding technique (18,19) and by a growth hormone by a double antibody radioimmunoassay (20).

To assess the time course of hormonal response, rats were cannulated by a modification of the method of Brown and Hedge (21), under pentobarbital anesthesia. Polyethylene tubing (ID = 0.034", OD = 0.050", Intramedic PE-90) was inserted into the jugular vein to the level of the superior vena cava. The other end of the tubing was exteriorized behind the head, where it is least accessible to the rat. By attaching an extension tube (ID = 0.023", OD = 0.038", Intramedic PE-50) to the cannula on the day of the experiment, the investigator could withdraw 0.2-0.3 ml of blood while remaining outside the exposure chamber. Samples were obtained at 15-30 minute intervals during and after the exposure without disturbing the rat.

RESULTS

Cranial exposure of the anesthetized dog to incident power densities of 20 mW/cm^2 to 100 mW/cm^2 for 1 hour revealed that body temperature increases can occur at sites other than within the electromagnetic field (11,12). In general, microwave exposure slowed or reversed the anesthesia-induced cooling process within the body during exposure. Of the various body temperatures monitored, the tympanic temperature is the most sensitive indicator of small temperature changes during this type of exposure. Exposure to 60 mW/cm^2 demonstrated the existence of body temperature gradients and changes in these gradients in a 21° C ambient environment (Table 1). In addition, increased cooling rate of the extremities was observed during exposure. As this latter effect is not dependent on exposure, this is interpreted as evidence of body heat regulation mediated by altered blood flow to the extremities. The rate of absorbed microwave energy when the cranial area was exposed to 60 mW/cm^2 was roughly equivalent to the rate of heat loss at 21° C ambient as a result of anesthesia-induced cooling. Growth hormone and serum thyroxine (T_4) levels did not seem to be affected by the exposures.

Table 1. Body temperature gradients in dogs cranially exposed to 2450 MHz (CW) microwaves at 60 mW/cm^2 for 1 hour

Dog	Mean Slope* 0-60 min.	Mean Slope** 60-120 min.	Slope Change From MW Exposure
5685, exposure	- 0.66	- 0.12	+ 0.54
6177, sham exposure	- 1.86	- 1.80	
exposure	- 1.68	- 0.18	+ 1.5
6191, exposure	- 0.54	+ 0.06	+ 0.60
6196, sham exposure	- 1.02	- 1.02	
exposure	- 0.60	0	+ 0.6
6197, sham exposure	- 0.12	+ 0.24	
exposure	- 0.18	+ 0.72	+ 0.90
exposure	- 0.54	+ 0.60	+ 1.14

* Control period.

** Microwave exposure period.

Mean slope, in degrees C per hour, is an average of the slopes for each of the 7 measured temperatures: rectal, tympanic, skin (fore paw, hind paw, groin, axilla, back).

In dogs, localized thyroid exposure revealed a significant increase in thyroid (T_4 and T_3) secretion rate in the absence of heating of other parts of the body (13,14,15). Figure 1 shows some typical thyroxine secretion rates relative to thyroid temperature. The colonic and unheated thyroid temperatures remained relatively constant while the heated thyroid temperature closely followed the increases in applied microwave power. An increase in T_4 release rate to twice the equilibration period value was noted when the thyroid temperature rose to 38°-39° C. This response closely followed the exposed gland temperature; at 40°-42° C and 44°-46° C, T_4 release rate peaked 50 minutes into the exposure period at three times the equilibration value and about six times the equilibration period value respectively and then fell off rapidly. In these studies, the T_3 hormone release rate paralleled that of T_4. The specific absorption rate in the thyroid necessary to cause a 2° C temperature rise is estimated to be 58 W/kg or approximately four times the metabolic rate of dog thyroid. The effect of localized microwave heating appears to be a result of thermally induced increase in the metabolic rate of the thryoid gland itself without the interplay of other body systems (13,14).

Whole-body exposure of 6-8 week old unanesthetized rats revealed the interrelationship of power density, duration of exposure, colonic temperature increase, and corticosterone level. This information is summarized in figures 2, 3 and 4. Essentially, the results of these studies show that colonic temperature increased at power densities of 13 mW/cm^2 or greater and was proportional to the power density, as well as the duration of exposure (Fig. 2). The mean colonic temperature of rats exposed to 13 mW/cm^2 for all three exposure durations was significantly higher than the mean colonic temperature of the corresponding control group. Up to 40 mW/cm^2, there was very little difference in temperature response between 30- and 60-minute exposures. At 50 mW/cm^2 and 60 mW/cm^2, however, 60-minute exposure resulted in a considerably greater temperature rise than at 30 minutes ($P < 0.01$). At power densities > 30 mW/cm^2, 120-minute exposure resulted in larger temperature increases than 30- or 60-minute exposures at the same power density.

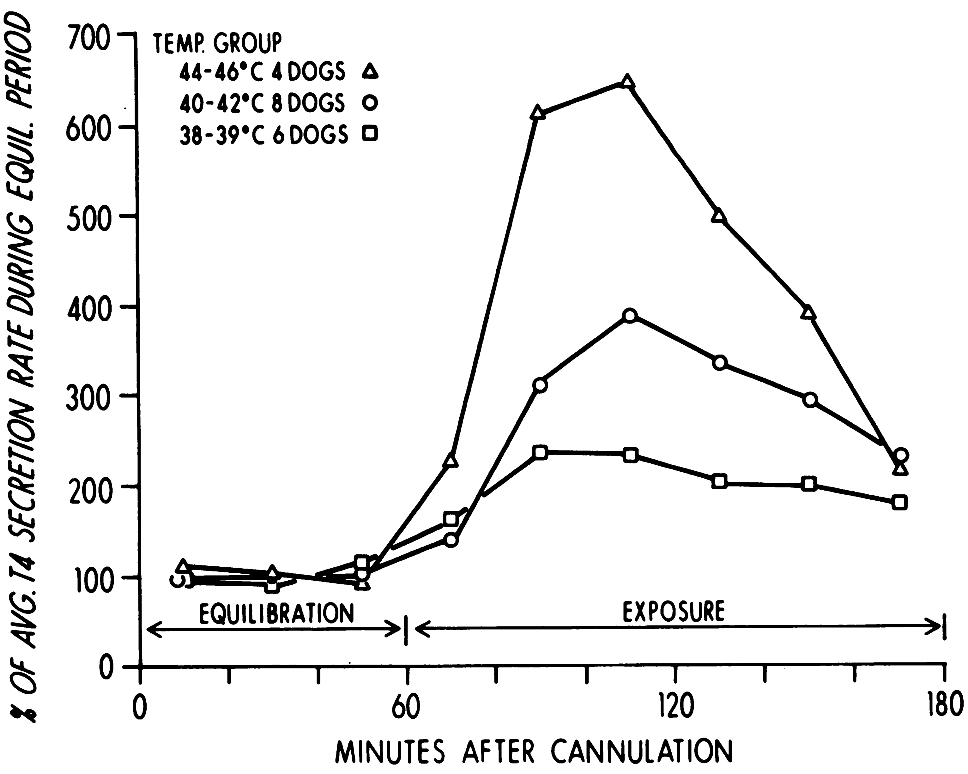


Figure 1

Plasma corticosterone levels exhibited a variable power-density/threshold pattern of response, with a different threshold for 120-minute exposures than for 30- or 60-minute exposures. For 30- or 60-minute exposures, no significant increase over control levels was observed below exposures of 50 mW/cm^2. Statistically significant ($P < 0.01$) increase over control values was evident at 50 mW/cm^2 (Table 2). For 120-minute exposures, corticosterone levels were significantly increased ($P < 0.05$) at 20, 30 and 40 mW/cm^2. For all three durations of exposure, a strong correlation was evident between mean colonic temperature and mean plasma corticosterone levels (Fig. 4).

Plasma corticosterone levels in acutely hypophysectomized rats exposed to 60 mW/cm^2 for 60 minutes were below control levels. Other groups of rats were pre-treated with dexamethasone before being exposed to 50 mW/cm^2 for 60 minutes. The corticosterone response to microwave exposure was completely suppressed by 3.2 µg dexamethasone/100g body weight (16,17).

In studies of this nature it is possible that corticosterone levels may vary during exposure that may not be evident when terminal (sacrifice) data are used. The use of cannulated rats provided an insight into the time course and fluctation of the corticosterone response. The results of these studies are shown in figure 5. Within 15 minutes, at exposure of 30, 40, 50 and 60 mW/cm^2, the corticosterone concentration rose to levels comparable to those following other types of stimuli. Analysis of the data revealed that corticosterone increase is related to both the power density and duration of exposure as shown in table 2. It was also apparent that the plasma corticosterone levels stabilized after 30 minutes at 40 mW/cm^2, increased at 50 and 60 mW/cm^2, but showed a decrease after 15 minutes at 30 mW/cm^2. At termination of exposure corticosterone returned to basal levels within 30 minutes.

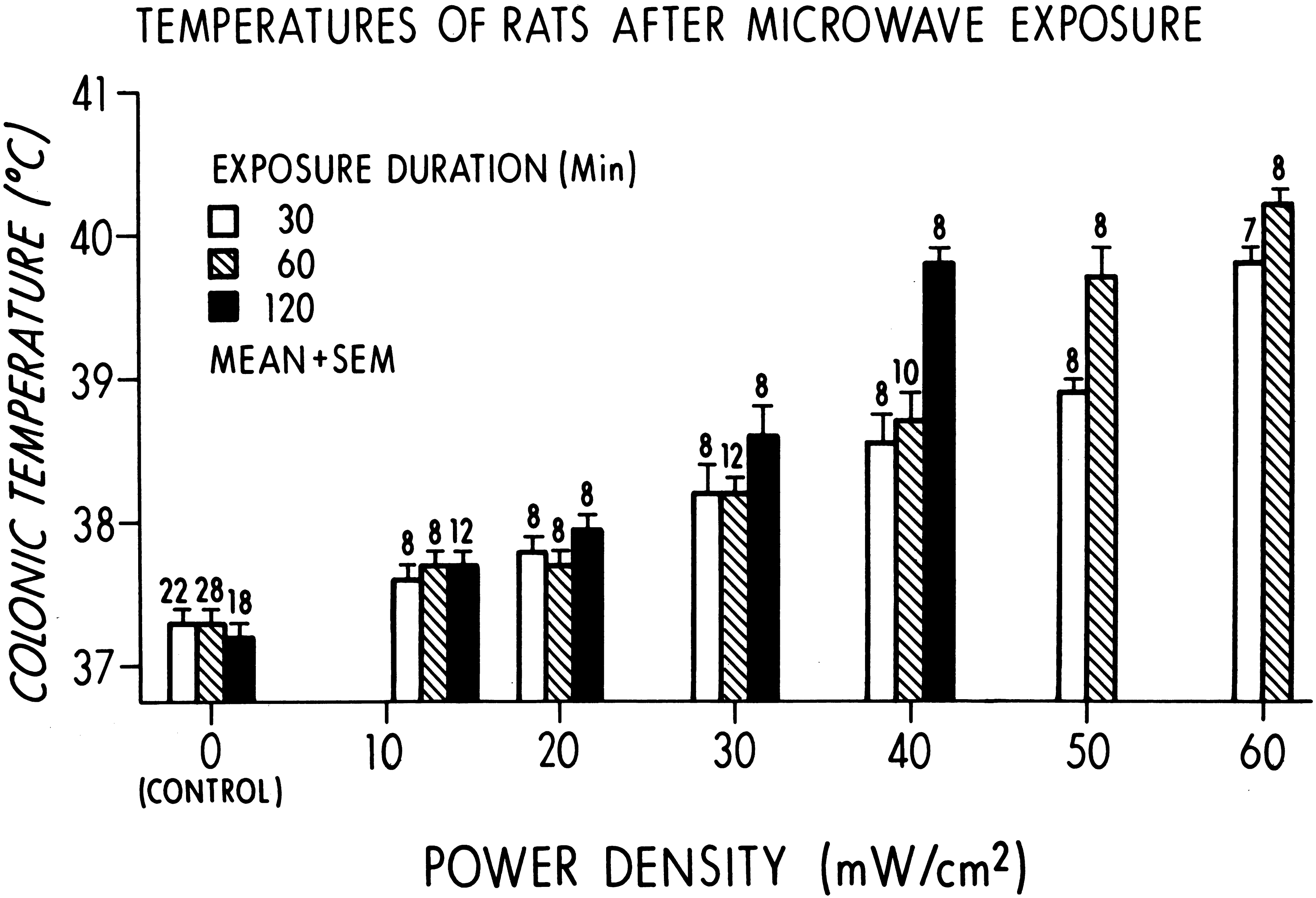

TEMPERATURES OF RATS AFTER MICROWAVE EXPOSURE
COLONIC TEMPERATURE (°C)
41
40
39
38
37
EXPOSURE DURATION (Min)
□ 30
▨ 60
■ 120
MEAN + SEM
22 28 18
8 8 12
8 8 8
8 12 8
8 10 8
8 8
7 8
0
(CONTROL)
10
20
30
40
50
60
POWER DENSITY (mW/cm²)

Figure 2

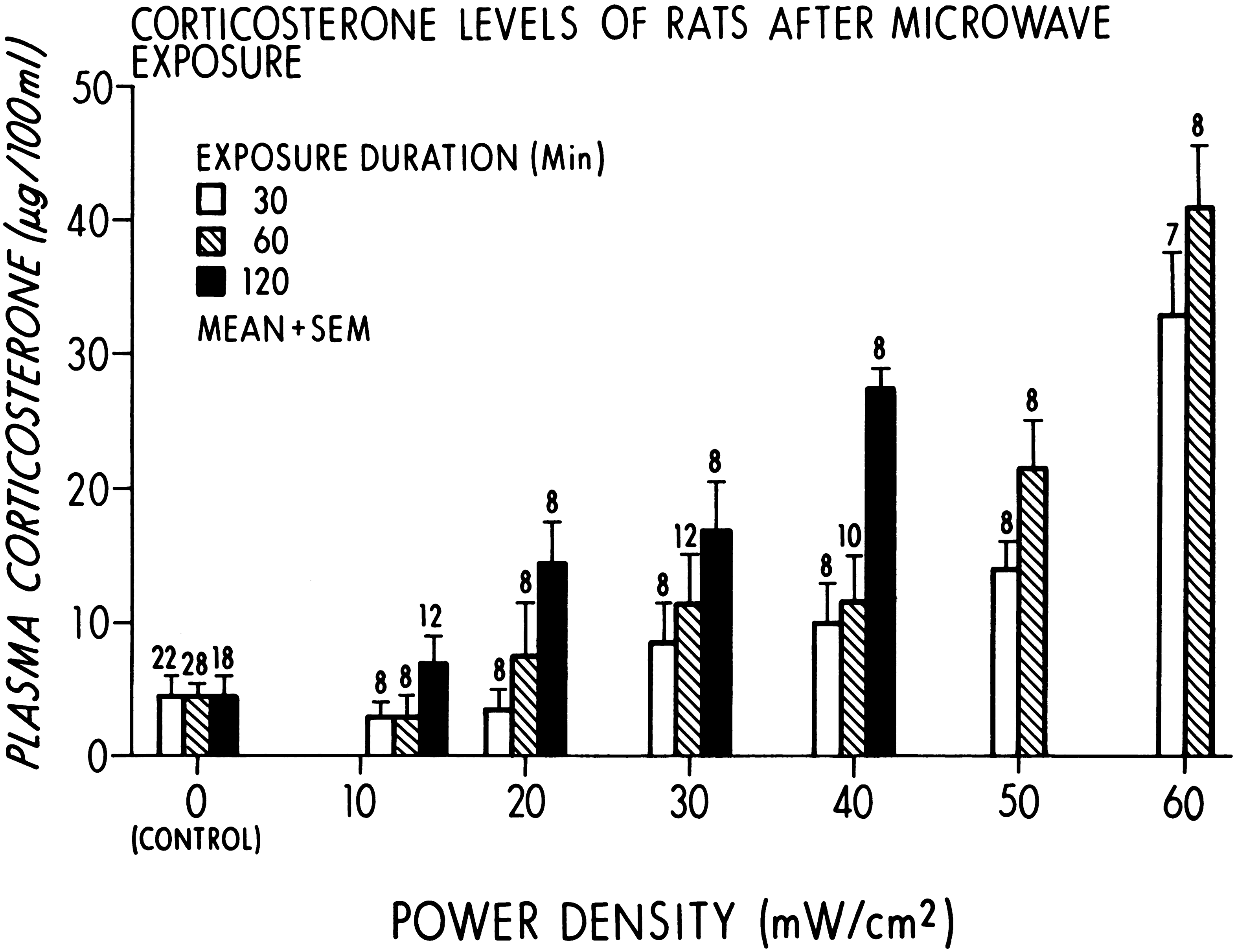

CORTICOSTERONE LEVELS OF RATS AFTER MICROWAVE EXPOSURE
PLASMA CORTICOSTERONE (μg/100ml)
50
40
30
20
10
0
EXPOSURE DURATION (Min)
□ 30
▨ 60
■ 120
MEAN + SEM
22
28
18
8
8
12
8
8
8
8
12
8
8
10
8
8
8
7
8
0
(CONTROL)
10
20
30
40
50
60
POWER DENSITY (mW/cm2)

Figure 3

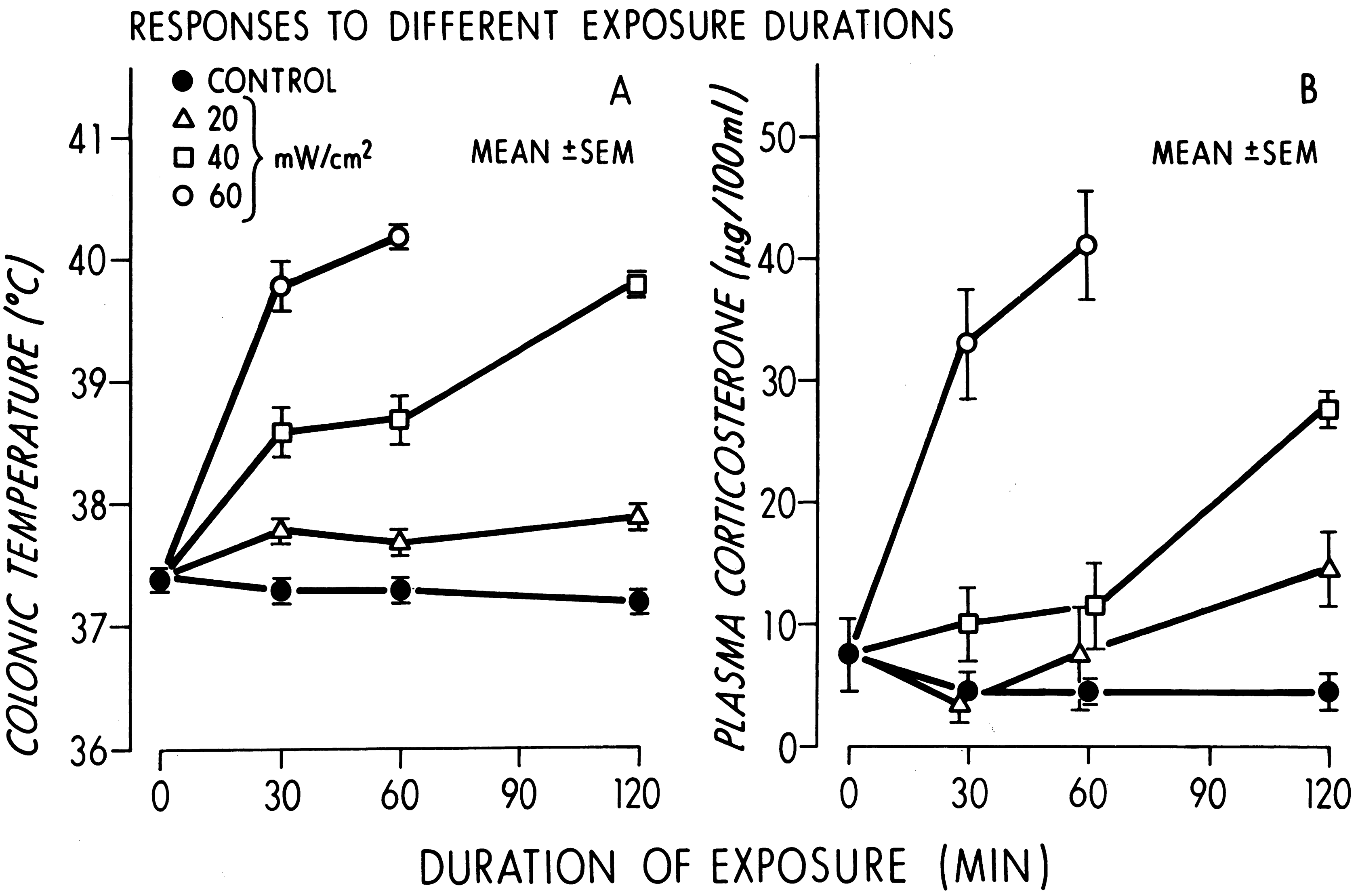

RESPONSES TO DIFFERENT EXPOSURE DURATIONS
● CONTROL
△ 20
□ 40
○ 60
mW/cm²
A
MEAN ±SEM
COLONIC TEMPERATURE (°C)
41
40
39
38
37
36
B
MEAN ±SEM
PLASMA CORTICOSTERONE (μg/100ml)
50
40
30
20
10
0
0
30
60
90
120
0
30
60
90
120
DURATION OF EXPOSURE (MIN)

Figure 4

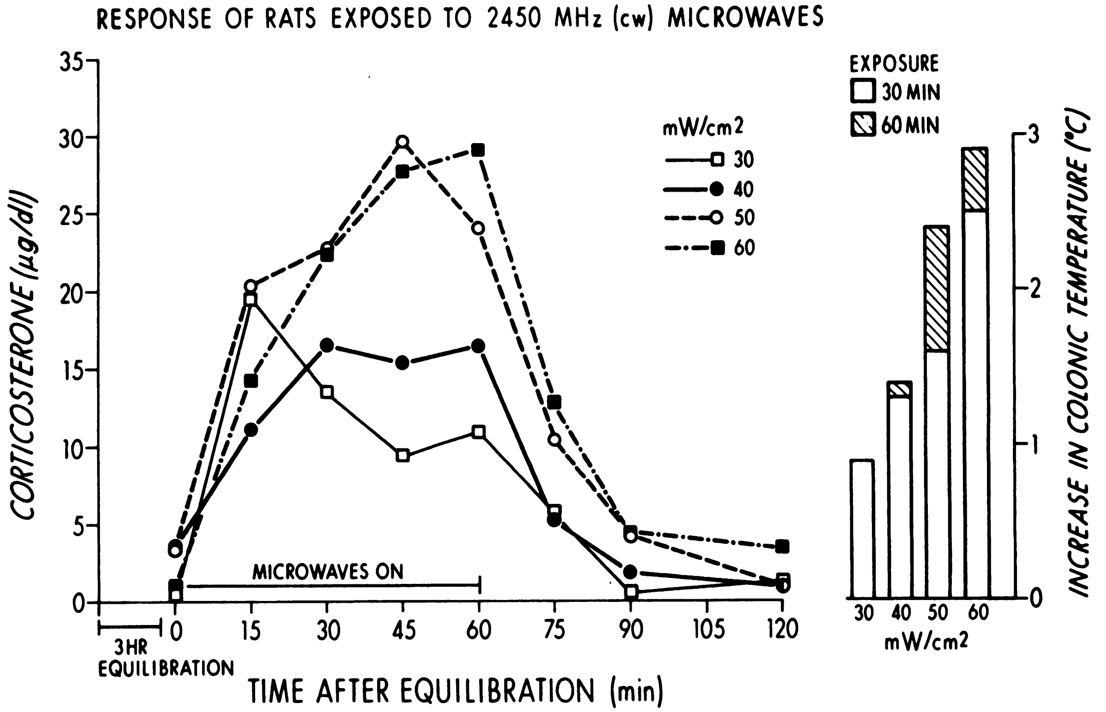


Figure 5

Table 2. Increase in Corticosterone Level in Rats Exposed to 2450 MHz (CW) Microwaves

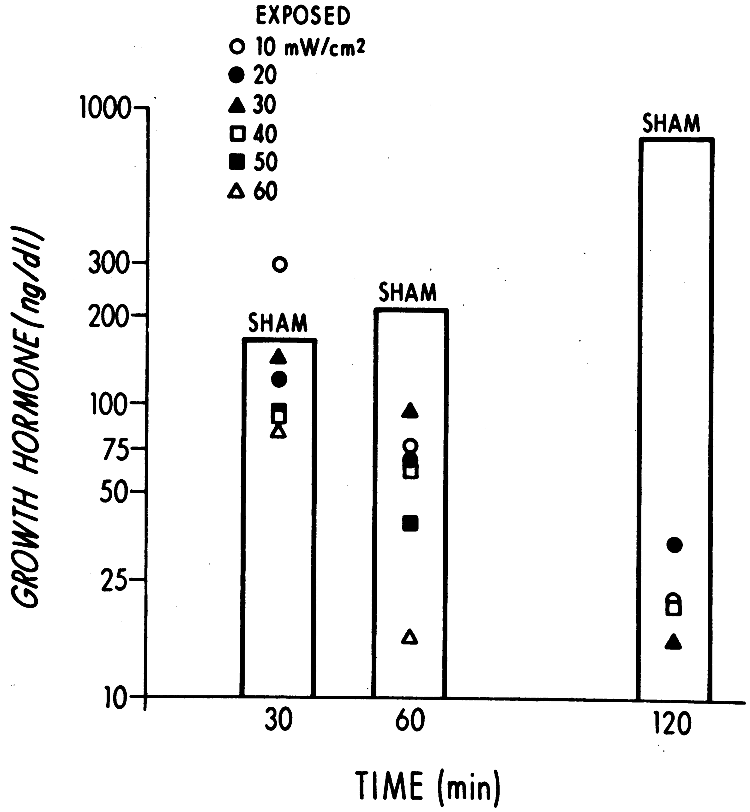
Power density (mW/cm^2)	Duration of Exposure 30 min	60 min
30	1.8*	2.7
40	2.2	2.6
50	3.1	5.0
60	7.2	9.6

* Ratio of corticosterone level in irradiated/sham exposed rats.

Growth hormone (GH) levels reflected the stress effect of microwave exposure as indicated in figures 6 and 7. In the absence of microwaves, the animals were very inactive, usually curled up and appeared to be asleep. Of interest is the progressive decrease in GH level at each power density as the duration of exposure is prolonged (Fig. 6). Sequential sampling permitted examination of the time course of GH decrease during exposure, and the return to higher levels shortly after exposure was terminated. Figure 7 depicts this response in rats exposed to 30 mW/cm^2 and 60 mW/cm^2.

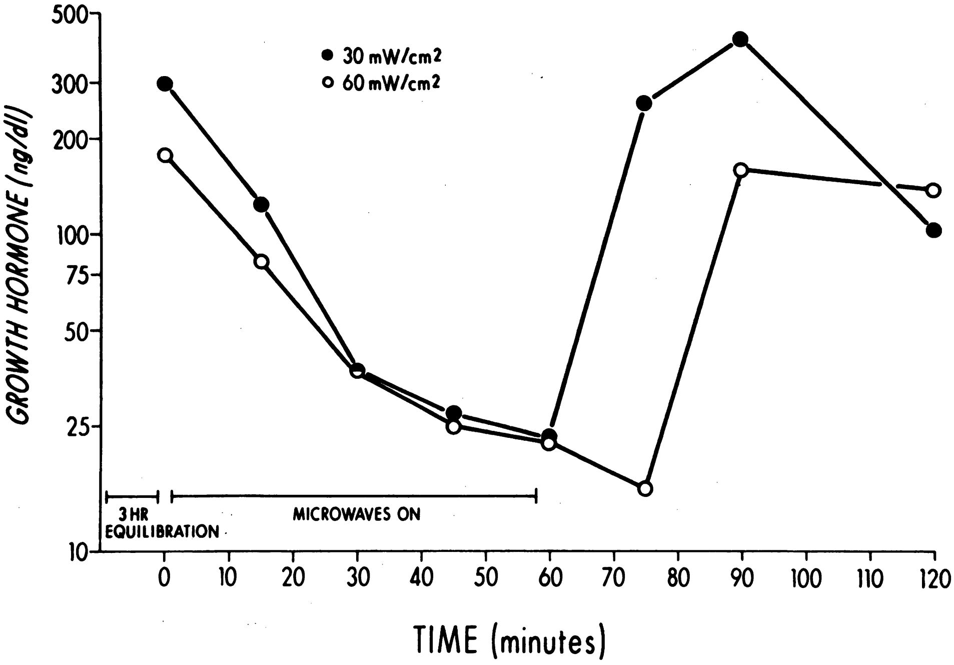
DISCUSSION

These studies show that temperature and endocrine perturbation can occur in rats and dogs acutely exposed to certain power-density/time durations of 2450



GROWTH HORMONE RESPONSE IN RATS EXPOSED TO 2450 MHz (cw) MICROWAVES

Figure 6



GROWTH HORMONE RESPONSE IN RATS EXPOSED TO 2450 MHz (cw) MICROWAVES

Figure 7

MHz CW microwaves. Endocrine response, however, is related to increase in temperature of the body or the individual endocrine gland and is of a transient nature.

Exposure of the head alone demonstrates the existence of readily measured body temperature gradients and significant changes in these gradients during anesthesia with and without head exposure to microwaves in a 21° C ambient environment. Temperature differences of up to 2.9° C can be measured in the anesthetized dog. These observations and conclusions suggest the importance of body temperature gradients in any study of the effects of microwaves (11,12).

Localized microwave heating increases the release rate of thyroxine and triiodothyronine from the thyroid. This increase is independent of stimulation from the hypothalamus and pituitary gland. Therefore, increases in thyroid gland function observed in man and animals exposed to microwaves cannot be assumed to result exclusively from hypothalmic-pituitary interactions. Microwave or radiowave frequency exposure which results in local neck heating of 2° C or more stimulates thyroid gland secretion. The specific absorption rate in the dog thyroid gland necessary to cause a 2° C temperature rise is estimated to be 58 W/kg or approximately four times the metabolic rate of dog thyroid tissue (13,14,15).

The time course of the response of the thyroid gland suggests a mechanism of stimulation. The plateau in hormone secretion rate observed when the gland temperature is 38-39° C indicates continuous stimulation consonant with a general increase in the gland's metabolism, probably caused by heating. The peak and decrease in hormone secretion rate observed at gland temperature of 40-46° C is suggestive of an initial transient increase in metabolism followed by either thermal damage to the biochemical systems of hormone secretion or a depletion of the readily releasable supply of hormone. A direct thermal stimulation of the thyroid has thus been demonstrated. This stimulation shows one mechanism of action of microwaves on thyroid gland function.

In previous studies we showed that in the young rat (8-12 weeks of age) exposed to microwaves, growth hormone response is altered at various power densities (4). At 10 mW/cm^2 there is an increased level of growth hormone; whereas, at 40 mW/cm^2, rat serum growth hormone drops after 60 minutes of exposure to significantly low levels. These results support the concept that exposure of rats to high (thermogenic) levels of microwaves for periods of 1 hour or longer represents hypothalamic-hypophyseal responsiveness, no doubt as a result of the thermal burden to the animal.

The response of the hypothalamic-pituitary-adrenal (HHA) axis to nonspecific stress has been well documented in the physiological literature of the last 30 years. Many different stimuli, including ether anesthesia, restraint, injection, surgery, fracture, and extreme cold, cause a rapid and qualitatively similar increase in pituitary ACTH secretion that causes a rise in adrenal corticosterone secretion in the rat within minutes of the onset of a stressful stimulus. The adrenal axis response is one of the first physiological mechanisms to react to a stimulus that disrupts the normal homeostasis of the animal.

By sequential sampling it has been shown that plasma corticosterone increases within 15-30 minutes of the start of exposure and (perhaps more significantly) falls sharply within 15-30 minutes after termination of exposure. The response to microwave exposure is transient in all cases. These results suggest a complex interplay between stimulatory and inhibitory influences on the HHA axis, depending upon the intensity and duration of the hyperthermia.

Investigations into the response of the adrenal axis to acute microwave exposure reported in the literature in the past have been inconclusive, with some indications of either stimulation or inhibition of the pituitary-adrenal axis by microwave exposure to different power densities. To carry out such studies, one has to be cognizant of the important contributions of biological factors such as circadian rhythms, as well as physiologic and nutritional equilibrium of the experimental animal. In addition, the concomitant influences of physical factors such as heat, light, noise, etc., have to be considered. One also cannot ignore the possible psychosocial interactions among experimental animals and between the experimental animals and man, whether it be the investigator himself or the persons who care for the animal. It is important that these considerations be included in any study of the biologic effects of microwaves. In addition, the question as to whether the biologic system can recover its initial state after perturbation is an essential part of such studies.

The evidence obtained in these experiments is consistent with the hypothesis that the stimulation of the adrenal axis in the microwave exposed rat is a systemic, integrative process as a result of a general hyperthermia. The results indicate that the microwave-induced corticosterone response observed in rats is dependent upon ACTH secretion by the pituitary, i.e. the adrenal gland is not directly stimulated by microwave exposure. Adrenocortical function may be a sensitive, quantifiable indicator of the physiologic compensation required of an animal exposed to microwaves.

It is apparent that acute microwave exposure at a high enough power density stimulates the adrenal axis. This stimulation does not occur, however, until power densities are greater than those required to cause a significant increase in temperature of the rats. The extent of both the colonic temperature rise and the rise in circulating corticosterone levels is increased by exposure for a longer time. The range of 20-30 mW/cm^2 seems to be a transitional area for stimulation of pituitary-adrenal activation in the rat. The response appears to be nonspecific and shows a close correlation with temperature. Apparently the increased heat burden imposed on the rat by the microwaves is the important stimulus in this adrenal axis activation. The observation that the longer times of exposure lead to increased temperature and pituitary-adrenal activation supports the concept that the thermal burden is the factor in these responses. In trying to relate these findings to man, one has to invoke physiologic and physical scaling factors. Such scaling factors are required because of interspecies' differences in thermal regulation and body size.

CONCLUSIONS

1. Endocrine changes in dogs and rats as a result of exposure to 2450 MHz (CW) microwaves are related to temperature increase.

2. Exposure of rats to 2450 MHz CW microwaves at thermogenic power-density/time durations can result in changes in plasma corticosterone, growth hormone and thyroid hormone levels.

3. The effects are transient, disappearing within 15-30 minutes after 1-2 hour exposure.

4. Power density of 20-30 mW/cm^2 appears to be the transitional range for pituitary-adrenal activation in the rat.

ACKNOWLEDGEMENT

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OPEN DISCUSSION

DR. SESSION: Being new at this business and trying to pick up as much as I can about methodology, I am sensitive to many of the questions and responses I hear from people who seem to know what they are doing. I hear a term quite frequently: multi-animal exposure--it seems to be something that is analogous to the T-Test fallacy and is to be avoided at all extremes. I notice that you seem, at least in your arrangement, to be making an economical use of space and time by radiating four animals at a time. The multiple irradiation is usually beneficial to the experimental paradigm; but I wonder, is it necessary to check the data with animals irradiated individually? I am sure you probably did that with the animals that were cannulated. Have you ever seen any differences between the two that suggest interactions that should be avoided, and how does one go about doing this?

DR. MICHAELSON: I am very glad that you asked that question. You may remember, in one slide we pointed out that we have a 4-inch separation between cages. These cages were mapped previously, with animals in them, to make sure that there is uniformity from one cage to another. Unless you do that, you could run into trouble. It is very definitely known that there is a tremendous animal-to-animal perturbation. If you separate them by certain factors of the wavelength of the energy you are using, you can overcome the variation.

DR. SESSION: You can feel confident that you have overcome the variation?

DR. MICHAELSON: If you measure it and know what you are doing, I feel confident that you will overcome it.

DR. SESSION: I also would be interested in hearing the observations that you promised to make about the necessity for habituating animals to handling, habituation of the temperature response, and so forth.

DR. MICHAELSON: Yes, however, I do not happen to have those particular slides with me. If you are going to do stress studies, any kind of physiologic study, or a study that may pertain to many other biological functions, it is very important that an animal be adjusted to his environment and to the animal-person interaction. I imagine that Don Justesen can say a lot more about this than I can. It is well known that it takes at least 10 days for these animals to adjust to a new environment; Mikolajczyk, in Poland, showed this. We have shown it also, and it is very real; the temperature response is not stabilized; that is, normal temperatures--circadian rhythms--are not stabilized; hormonal levels are not stabilized; and the corticosterone increase, a very labile thing, is not regular between 1 and 2 weeks after arrival in a new environment. It must be a tremendous disruption for that poor rat to be moved from one environment to another. You, therefore, have to be very careful; these animals have to be played with, handled, and gentled.

DR. ALBERT: Did you do any correlative studies on the releasing factors for the growth hormone and the adrenalcorticotropic?

DR. MICHAELSON: We never did growth hormone releasing factors. We have some studies that have been completed; however, it is under another contract (that is why I did not report it), in which we used median emminence and CRF. There are some very interesting results. We hope to apply the results to microwave exposure under the longer durations. First though, we must get these 8-hour protracted exposures and find the periodicity of the corticosterone. As you know, around 2 o'clock in the afternoon they always go up and we are trying to relate that. In other words, we are going to repeat some of these studies, I hope someday, in the late afternoon. I believe that is another point that Don Justesen tried to make this afternoon. I feel it will make a difference if we expose these animals in the afternoon as opposed to what we have done in the morning, because the corticosterone levels are high at that time.

DR. ALBERT: It would be nice to know what releasing factor correlates with the actual circulating hormones. Did you look at any adrenal morphology in terms of subcellular appearance of organelles in the cytoplasm of these corticol cells.

DR. MICHAELSON: No, I did not.

DR. ALBERT: Did you have any question in mind as to the sex and the stage of the estrocycle when you put the animals in the cage?

DR. MICHAELSON: That is an excellent question; I am glad you asked. I forgot to mention that these were males.

DR. POSTOW: I would like to point out the fact that was made this morning: the NARDA 8300 averages over a significant volume and, therefore, will erroneously give you the same reading in one cage versus another; whereas, the reflected field of the animal will vary quite significantly over that space that the animal is occupying. For example, the reflected field of a mouse may extend to a wavelength from the mouse and vary by a factor ± 50 percent; whereas, if you measure it with an averaging probe like the NARDA, you will not see this at all.

DR. MICHAELSON: I do not understand. If these cages are separated by 4 to 6 inches, and you measure each one individually without the rats and then with the rats in them, I do not understand why you can not see if there is any perturbation of one rat on the other.

DR. POSTOW: The perturbation caused in the adjacent cage, by a rat in the second cage, will set up a field barrier and will set up a standing wave in the adjacent cage. There will be a different field from one position in the cage to the other.

DR. MICHAELSON: There will also be a different field as the animal turns and moves around in the cage.

DR. POSTOW: Yes.

DR. MICHAELSON: I am sure that these are real problems; it is not as simple as that.

DR. CARPENTER: You say that this is a stress reaction and that the stress reaction corticosterone increase can be evoked by cold and by various stresses. I have two questions: can you imagine what will happen if you apply microwaves but keep the animal hypothermic? And secondly, why not try it on a hypophysectomized rat?

DR. MICHAELSON: Yes, we have done it.

DR. CARPENTER: And what happened?

DR. MICHAELSON: In a hypophysectomized rat you do not get the corticosterone response.

DR. CARPENTER: My other point was on the thyroid. If I saw the slide correctly, the concentration decreased partway through the exposure; but in the others, it cut down immediately at the termination of exposure.

DR. MICHAELSON: That is a very good observation. On the corticosterone, we show the very rapid decrease. The thing you saw was the thyroxine release from the dog thyroid; however, somewhere midway in the exposure it starts dropping down. It may be because of utilization of some enzymatic process or some degradation effect, but that is a very interesting observation that it decreases. I do not think this is a nonsignificant effect. In other words, the animal is not adapting to the microwaves under those conditions; I think it is the utilization of some substrate.

DR. JUSTESEN: When you were cannulating the animals in the field and drawing fluid out....

DR. MICHAELSON: We did not canulate them in the field; they were already canulated.

DR. JUSTESEN: Alright, I was thinking of the hose going to the animal's head as being an extension of the cannula in the same. Whatever the nomenclature, do you think the act of drawing that electrolyte fluid out while the field is on causes any chance for perturbation in one way or the other?

DR. MICHAELSON: These are saline filled?

DR. JUSTESEN: Yes, saline has pretty good conductive properties.

DR. MICHAELSON: There is no change in the fluid content of the cannula?

DR. JUSTESEN: No, I was just wondering if it would warm or heat and if that in anyway would cause confounding? I am just making a methodological observation. If you have a line, a conductor formed by a conductive fluid in a field over a long period of time, will there be a significant capture of energy?

DR. MICHAELSON: I see what you probably are suggesting. When you work with these cannulas, you do not draw the blood that is in the cannula at the time; you have to evacuate that cannula and inject saline.

DR. JUSTESEN: So it is moving fast during the time of the draw?

DR. MICHAELSON: Otherwise you have some blood that may be clotted at one point. You inject saline through the cannula until it is clear again, and then you pull the blood out; therefore, the blood that is in the cannula--that you are drawing out--is not the blood that is irradiated. Is that the question you were asking?

DR. JUSTESEN: Also, this is the role of the head, in the sense that the psychologist would use it, in the results you are getting. I almost want to reach out and grab for the interpretation because of this long time of equilibration you are talking about. If you had run suitably anesthetized controls, you would not have seen these differences yourself. Would you speculate on that? Are the animals being stressed? Is your rat saying, "I'm upset and so I'm going to set all kinds of neuroendocrine things into play"? Or is this strictly a systemic response?

DR. MICHAELSON: No, if I understand your question correctly. You do see fluctuations, not as marked as this of course; but those earlier fluctuations that we saw, in temperature and corticosterone level, you see this throughout the period of the day. The fluctuations are part of the circadian rhythm. I am not sure if this is what you are asking, but I think that this is the point that you may have tried to make earlier. If you look at the exposure for 1, 2, 4, and 8 hours, follow the colonic temperature, you see fluctuations throughout the day, and you see some relationship to power density. If you look at zero power density, you find that there is a fluctuation throughout that period of the day. And if you carry it through the night, you will see another fluctuation; of perhaps, two or three cycles. With corticosterone (we now have corticosterone growth hormone TSH data under these conditions) you see a similar flucuation, but we have not characterized it.

DR. JUSTESEN: Yes, I note that in some of those cases, the curves are "U" shaped, and I was thinking of Henry Ho's data that has been variously interpreted as an avoidance response. His curves are sort of "J" shaped over the same period of time. If you remember, the slides he had on earlier today started out higher; went down; and were coming up just before the period of termination or the time of termination of the experiment.

DR. MICHAELSON: Yes. Here is an example where you almost get a trough, a "U" shaped curve. This shape is an inversion, of course; because, here you are throwing in some sort of stimulus to the animal, whatever it is. Even though it is only 10 milliwatts, it is doing something to the animal on top of his periodicity. So this may be an artifact; these changes could easily be something built on top or overshadowed by circadian periodicities.

DR. JUSTESEN: Perhaps then as that Dutch physician who wrote the book on "The Body Has a Head," we are discovering that all over again.

THE EFFECT OF REPETITIVE PRENATAL LOW-LEVEL MICROWAVE EXPOSURE ON DEVELOPMENT IN THE RAT

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ABSTRACT

Twenty-four time-mated Sprague Dawley rats were exposed to low-level 2450 MHz microwave radiation daily for 5 hours from day 3 through day 19 of gestation at an average exposure level of 10 mW/cm^2. Controls were simultaneously sham exposed. Exposed animals were positioned reproducibly in a square configuration so that 12 animals were parallel to the electric vector of the microwave field (group AC) and 12 animals were parallel to the magnetic vector of the field (group BD). Following sham or microwave exposure, animals were allowed to deliver normally. Litter size was determined at time of birth. Body and brain weight were determined on day 2, 3, 6, 7, 8, 9, 14, and 15 postpartum. Spontaneous postnatal mortality was observed and recorded. Litter size did not appear to be affected by the microwave exposure utilized in this experiment. Total number of dead offspring and the number of litters with mortality appeared to be higher for exposed groups versus the controls. However, no significance can be attached to this observation since the experiment was not designed to evaluate postnatal survival or mortality. Body and brain weight were consistently lower than control values for exposed animals oriented parallel to the electric vector of the microwave field.

INTRODUCTION

One of the areas of concern with regard to the biological effects of microwave radiation relates to the potential of such radiation to influence prenatal development. The prenatal period is probably one of the most sensitive in mammalian biological development. It is a period which can be disturbed by a number of stresses, including drugs (1), ionizing radiation (2), and hyperthermia (3).

Previous studies in our laboratory, reported by Rugh et al. (4,5) have examined the effect of acute, relatively high level microwave exposure during the period of organogenesis in mice. His investigation involved single exposure to 2450 MHz CW microwaves, at levels of 123 mW/cm^2, for 2-5 minutes, on the 8th day of gestation. Rugh reported a number of effects resulting from this microwave exposure, including gross hemorrhage, resorptions, exencephaly, stunting, and fetal death.

It remains unanswered whether repetitive daily exposures to lower levels of microwaves can affect mammalian development.

This is a preliminary report of a study designed to extend the observations made by Rugh to a related rodent, the rat. This study also employs 2450 MHz microwave radiation, but it differs from the previous investigation in that it probes the effect of a substantially lower level of exposure, which is administered repeatedly during the course of prenatal development.

MATERIALS AND METHODS

Time-mated Sprague Dawley rats obtained from Blue Spruce were used in this study. Arrivals were housed in individual cages, marked, and divided into control and experimental groups.

During microwave exposure, which takes place in an anechoic chamber, animals are housed in a Lucite container with removable top and bottom polystyrene grids which provide ventilation (Fig. 1). The Lucite containers are positioned reproducibly on a symmetrical square Lucite rack (Fig. 2) which is placed on a flat surface constructed of styrofoam and absorbent material. Each animal is assigned a position on the exposure rack and is always placed in that position during exposure. Animals are free to move about in the exposure container which has internal dimensions of 19 cm x 11.4 cm and is 7.6 cm high. The shammed animals normally move about in the container during the first 30 minutes of exposure, after which they settle down and become quiet at the bottom of the exposure containers. Frequently they are asleep at the end of the exposure period. Exposed animals appear to be more active during the period of exposure. A microwave truncated horn antenna is positioned 208 cm above the plane occupied by the experimental animals. The animal rack holds 24 animals at a time, 6 on a side positioned symmetrically. Twelve of the animals are oriented so that during the exposure they are parallel to the electrical field vector. These constitute group AC. Twelve are oriented so that during exposure they are parallel to the magnetic field vector. These constitute group BD.

Animals are exposed in the anechoic chamber at an average level of 10 mW/cm^2 for 5 hours per day, from day 3 to day 19 of gestation. Day 0 of gestation is the day on which female vaginal plugs are observed for indication of impregnation. The exposure period begins at 7:00 a.m. The anechoic chamber is illuminated during exposure. Control animals are treated in the same manner as experimentals, but are sham exposed at the same time at which experimental animals undergo microwave exposure. The exposure system has been previousy described (6).

The environmental temperatures in the anechoic and in the sham exposure chamber were not controlled. Thus variations in temperature were observed in the chambers during the course of sham and microwave exposure. Sham exposed animals were subjected to a mean temperature of 22.8 ±0.2° C while experimental animals were subjected to a mean temperature of 25.5 ±0.7° C. This pilot investigation provided an insight into the effect of this difference in ambient temperature during the course of radiation exposure, as will be seen.

While the current effort provides dosimetric information only in terms of average exposure within the containers housing the animals, we are presently preparing to conduct dosimetry to provide information on absorbed dose in tissue equivalent phantoms, and we will also examine energy deposition patterns both in phantoms and animal carcasses to provide an insight into dose distribution in tissues using thermographic techniques.

Following termination of exposures or sham exposures, animals were permitted to deliver normally.

Animals were taken from shammed and exposed litters on day 2, 3, 6, 7, 8, 9, 14, and 15 postpartum for determination of body and brain weight. Control and experimental litters were matched and equalized at time of first sacrifice. However, for this first study rigid criteria were not used to exclude litters which did not meet a specific number criterion at birth.

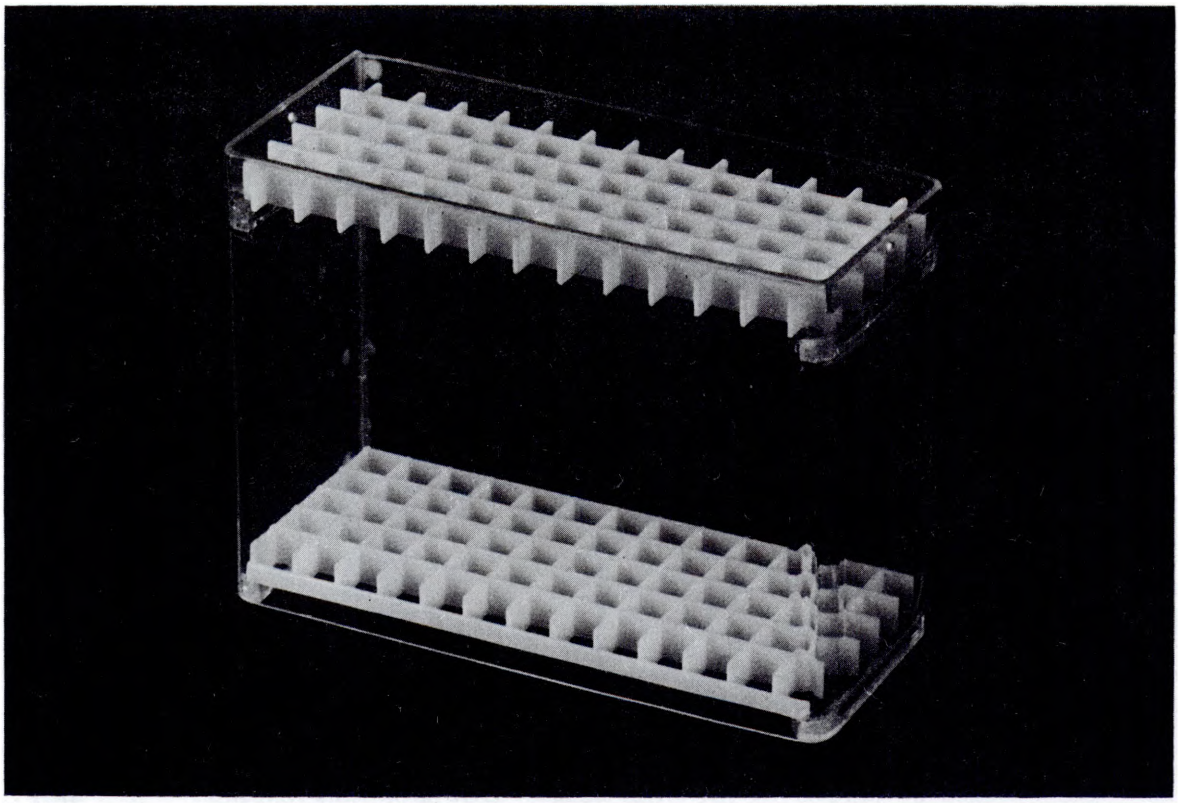

Figure 1. Individual Lucite animal container with open grid top and bottom. Dimensions are 19 cm x 11.4 cm and is 7.6 cm high.

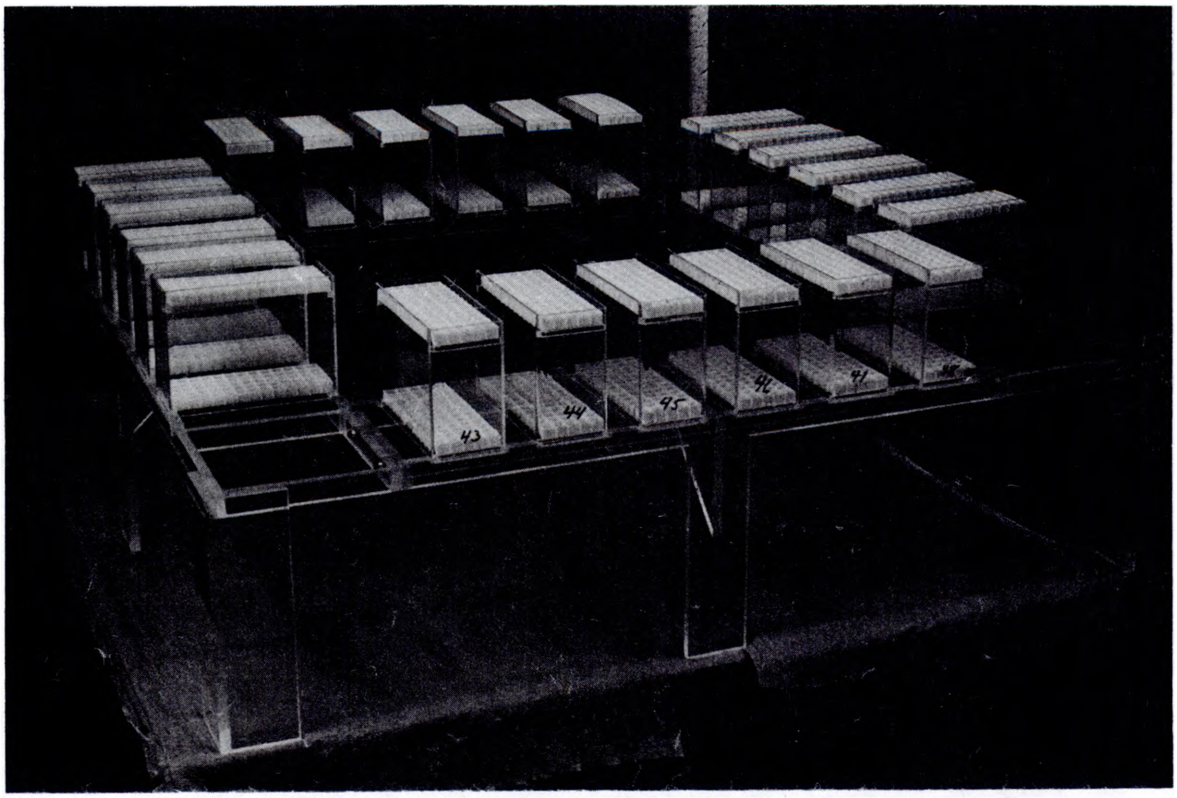

Figure 2. Animal holder support stand with holders in place used for repetitive exposures.

RESULTS

Litter Size

Table 1 presents the data for litter size determined at birth. No significant differences were observed between control and irradiated animals ($p > .05$ using t-test). Twenty-three of 24 control animals were pregnant and delivered litters. The range of control litter sizes was 4-15. Twenty-four of 24 exposed animals were pregnant and delivered litters. The range of exposed litter sizes was 5-14.

Table 1. Comparison of average litter size for all controls to the average litter size of group AC (parallel to electric field vector) and group BD (parallel to magnetic field vector)

LITTER SIZE		
CONTROL		EXPOSED
9.78 ± 0.51 (n = 23)*	AC	9.33 ± 0.74 (n = 12)
	BD	10.75 ± 0.39 (n = 12)

*n = number of litters

Mortality

This study was not designed to obtain a critical measure of postnatal mortality, since newborn animals were serially sacrificed from each litter according to the experimental protocol. Nevertheless, it is interesting to note that higher mortality was observed in exposed animals than in controls.

Table 2 shows these values expressed in terms of numbers of newborn animals that died during the 15-day period of observation.

Table 2. Comparison of all dead control newborn to the number of dead newborn in group AC (parallel to electric field vector) and group BD (parallel to magnetic field vector). Mortality recorded for total length of experiment

OBSERVED MORTALITY (Dead/Total)		
CONTROLS		EXPOSED
19/225	AC	17/112
	BD	13/129

Table 3 gives the values in terms of number of litters in which mortality was observed during the 15 day period of observation.

Table 3. Comparison of all control litters in which mortality occurred to litters having mortality in group AC (parallel to electric field vector) and group BD (parallel to magnetic field vector)

OBSERVED MORTALITY (Litters with Dead/Total Litters)

CONTROLS	EXPOSED
5/23	AC 6/12
	BD 7/12

In both Table 2 and Table 3 it can be seen that exposed values for mortality are higher than the control values.

Body Weight

Body weight was determined in control and exposed newborn animals at 2, 3, 6, 7, 8, 9, 14, and 15 days of age. For purposes of this analysis individual newborn animals were not treated as independent observations. Rather, mean values were determined for newborn animals derived from each litter and these means were treated as a single observation. Table 4 presents the mean values for body weight.

Table 4. Comparison of body weight for all controls to body weight for group AC (parallel to electric vector) and group BD (parallel to magnetic vector). Values are the average for all animals weighed at each point

BODY WEIGHT (gms)

Day	2	3	6	7	8	9	14	15
Control	6.7	7.8	11.8	13.3	15.7	17.9	29.6	33.6
(S.E.)*	(± .2)	(± .2)	(± .3)	(± .6)	(± .5)	(± .7)	(±1.4)	(± 1.8)
(N)**	(12)	(10)	(12)	(10)	(12)	(9)	(12)	(7)
Exposed								
AC	6.6	6.8†	11.6	11.2	14.7	16.0	27.4	31.3
(S.E.)	(± .2)	(± .3)	(± .3)	(±1.3)	(± .6)	(±1.3)	(±1.6)	(±2.5)
(N)	(6)	(6)	(6)	(6)	(5)	(5)	(5)	(4)
BD	6.5	7.1	11.7	13.9	14.6	17.9	29.3	32.9
(S.E.)	(± .2)	(± .4)	(± .9)	(± .7)	(±1.3)	(± .9)	(±2.2)	(±1.9)
(N)	(6)	(6)	(6)	(5)	(6)	(5)	(6)	(5)

*S.E. - Standard Error of the mean
**N - Number of litters from which samples taken
† $p < .05$ using t-test

It is interesting to note that the mean values for body weight tend to be lower for exposed animals in group AC than for controls although the number of animals was too small to allow the demonstration of statistical significance except for day 3. It should be remembered that the AC group was oriented parallel to the electrical field.

Exposed group BD which was oriented parallel to the magnetic field showed no clear trend of difference from the values observed in the controls.

Brain Weight

Determinations of brain weight were also made and are presented in Table 5.

Table 5. Comparison of brain weight between total control and group AC (parallel to electric vector) and group BD (parallel to magnetic vector). Values are averages for all samples determined at each point

	BRAIN WEIGHT (gms)							
Day	2	3	6	7	8	9	14	15
Control	.282	.337	.530	.614	.696	.805	1.180	1.272
(S.E.)*	(±.006)	(±.007)	(±.008)	(±.013)	(±.011)	(±.019)	(±.019)	(±.024)
(N)**	(12)	(10)	(12)	(10)	(12)	(9)	(12)	(7)
Exposed								
AC	.280	.309†	.511	.560	.667	.745	1.138	1.232
(S.E.)	(±.016)	(±.008)	(±.010)	(±.031)	(±.014)	(±.037)	(±.020)	(±.033)
(N)	(6)	(6)	(6)	(6)	(5)	(5)	(5)	(4)
BD	.285	.311	.523	.632	.678	.796	1.156	1.233
(S.E.)	(±.008)	(±011)	(±.016)	(±.013)	(±.026)	(±.018)	(± .033)	(±.017
(N)	(6)	(6)	(6)	(5)	(6)	(5)	(6)	(5)

*S.E. - Standard Error of the mean
**N - Number of litters from which samples taken
† - $\rho < .05$ using t-test

The trend of brain weight data again suggests that exposed animals in group AC, which were oriented parallel to the electrical field tended to have brain weights lower than those of the controls, with a significant difference demonstrable on day 3. Animals in exposed group BD oriented parallel to the magnetic field showed no clear trend of difference from controls.

When the data is expressed in terms of relative body weight and relative brain weight, to allow comparison of control vs. exposed groups as a whole (Table 6), it can be seen that the values for AC are lower than control for both body and brain weight. It is interesting to note that value for AC is also lower than BD for both brain and body weight.

Table 6. Comparison of relative brain and body weights between all controls and all group AC (parallel to electric vector) and group BD (parallel to magnetic vector). Relative brain and body weights were determined 2 to 15 days postpartum

	Relative Brain Weight	Relative Body Weight
Control	1.000 ± .006	.993 ± .013
(N)*	(84)	(84)
Exposed		
AC	.950 ± .011†	.922 ± .020†
(N)	(43)	(43)
BD	.982 ± .010	.975 ± .021
(N)	(45)	(45)

*N - Total number of samples measured.
† - $\rho < .05$ using t-test

DISCUSSION

The data obtained from this study would suggest that exposure of pregnant rats to microwave radiation for 5 hours per day at an average power density of 10 mW/cm^2 from day 3 to day 19 of gestation may influence postnatal body weight and brain weight. This study also suggests that postnatal mortality following low-level repetitive microwave exposure during pregnancy should be carefully evaluated.

Exposed animals were subjected to an environment which was, on the average, 2.5° C higher than that of the shammed controls. A suggestion of decrease in body weight and brain weight was observed in animals oriented parallel to the electrical field. Animals oriented parallel to the magnetic field showed no clear trend of difference from the controls. Since both exposed groups were subjected to the same ambient temperature, ambient temperature differences of this magnitude may not be a significant factor affecting the observed endpoints. It may be possible that the difference in orientation is associated with a difference in energy absorption by the two groups of exposed animals. This will have to be examined in future work.

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OPEN DISCUSSION

DR. OSEPCHUK: In your last point, you said that there is a distinct difference between AC and BD despite the same environmental temperature. If you look at your results on mortality, apparently both AC and BD showed about the same thing, which suggests maybe it is the environmental temperature.

DR. SHORE: This is something that I think we will have to look into.

DR. MICHAELSON: You mentioned that you did not cull the rats to maintain uniform litter size. What was the litter size, do you remember?

DR. SHORE: Approximately 10.

DR. MICHAELSON: Was there a distribution of litters, of numbers? I would like to make a suggestion, if I may, and you probably know this anyway, that one of the most important factors in this kind of experiment is to maintain uniform litter size. We found that for the rat you really should cull them back to about 9 per litter and 8 is even better.

DR. SHORE: This is why I mentioned that we had not culled the litters in this particular study, but we plan to in the future applications of the study.

DR. MICHAELSON: Yes, because this could make a difference in weight response.

DR. SHORE: This is a pilot investigation. I think that the information coming out of this study was used essentially to provide the insight into just about everything that we want to do in the replications of this study.

DR. JUSTESEN: Did you look at litter size as a function of treatment, and were there any indications of teratology?

DR. SHORE: Yes, we did look at litter size--that was the first slide that I showed, and we observed no differences in litter size. However, we did not look for teratology; to do that we would have had to sacrifice the animals and take them prior to birth.

DR. MUMFORD: Did the handlers complain that the exposed rats were more aggressive than the non-exposed rats?

DR. SHORE: No, however, that is kind of interesting. There was a report that was presented by Ed Hunt from Navy, indicating that animals exposed repetitively during the course of studies on microwave radiation exposure showed increased aggression. There has been, of course, another study presented at this meeting, by Dr. Frey, indicating exactly the opposite reaction. We did not notice any aggressive changes in the behavior of the animals; they appeared to handle the same way.

DR. SESSION: Did you make any observations about the relationship between the offspring and the mother. For instance did you notice how many offspring were away from the nest at any given time and the attentiveness of mother to offspring between the irradiated controls.

DR. SHORE: No, these were not parameters that we were looking for in this study.

DR. HUNT: May I clarify one point: it was not aggression that was displayed by the animals; they were more irritable.

DR. SHORE: I am sorry; I should have said it that way.

FROM THE FLOOR: Dr. Hunt, would you clarify that, is an irritable animal less likely to bite than an aggressive animal?

DR. HUNT: No, irritable simply means that when you tickle it or stimulate it in some way or other, it responds in a particular way. Aggression, I would take to mean that the animal is in a situation where it sponsors any activity toward any object; the animal does not have to be stimulated to elicit the response. I do not think that is too important a distinction, actually.

I also have a question. In this type of experiment, you are following the course of development where the mother is tending to the animals and is being exposed and handled daily. Is it possible that if the mothers had not been handled daily and had been kept in their own cages that you would have seen some difference between these animals and the controls that were sham exposed?

DR. SHORE: I did not mention it because the number is very small, but we did have a number of cage control animals. The initial suggestion--again suggestion only, because the numbers are small--is that there are probably differences between cage controls and the handled controls. We observed no mortality in the cage controls, which is a little bit surprising in the young following birth. It may be that this is one of the things that we will have to evaluate. The real thing of interest, of course, is the difference between exposed animals and shammed animals, because what we want to isolate is the effect of the microwave treatment. However, I think we do get some useful information from the cage controls, and we probably will want to conduct those as well.

DR. MICHAELSON: This is not related to the study that was just reported; rather; it is related to the dialogue between Ed Hunt and Don Justesen. I do not think Ed Hunt should have let go of that very important point--the difference between aggression and irritability--as easily as he did. I think it is a very important difference, and I think we should not leave it on the record that we are not concerned about the difference. I am sure that you will agree with me that we should separate these two reactions. It is true that when these animals are exposed to microwaves, depending at what level of temperature increase you have, you find them more irritable--there is no question about it. You take these animals when they are trying to regulate and they are prone, and if you should pick them up they are irritable. They are not aggressive; they are irritable. There are certain stages wherein sometimes you find them more irritable at the lower temperatures than at the higher, because at higher temperatures they are more relaxed; therefore, there are some very subtle implications here.

I also would like to make one other point on this study of aggression. I decided at this meeting that I was not going to say anything. However, the first paper of this meeting, in my mind, was a very poor example of the way biological research should be done today. I wish Allan Frey were here. The idea of using pain as an indicator of aggression, to have tails wound around a bar with no standardization, I think is inexcusable. I think it reflects on us as biological investigators, vis-a-vis Humane Society people. I personally would like to criticize that kind of experiment; because, if you have one person doing it and we approve, then I think we are all to blame.

DR. SHORE: Let me just make one additional comment with respect to the question raised by Dr. Osepchuk. One of the reasons that we have gone to the elaborate control that we are installing in our anachoid chambers is precisely to allow us to isolate the possible effects that may be associated with this kind of ambient temperature change. We will have the facility to conduct experiments at various temperatures; so we will be in a position to check out this one point of ambiguity that you referred to.

DR. CARPENTER: Did you find that the power density in the 24 plasticboxes was consistent?

DR. SHORE: We mapped the inside of the chambers at three positions in each one. I am not satisfied with this as being adequate dosimetry, as you well know from the type of work that we normally do in the Division; but as an initial point of departure, we did that. The dosimetry mapping, by the way, was done with animals in every cage except the cage that was being mapped; what we saw, essentially, was a ± 10 percent distribution of values.

DR. SESSION: Regarding Dr. Michaelson's comments on some of the considerations for humane treatment of animals I do not think that we should leave it on the record that we, as biological investigators, are not concerned about the humane considerations of producing hypothermia in animals. I think that the comparison of a tail pinch methodology to producing severe hypothermia in trapped animals is a little bit hair splitting; and I, myself, am concerned about this as an investigator. I do not have any good answers for it, but I think as biological investigators we should be concerned about the total gamit of our experimental manipulations.

DR. LEACH: I have seen signs of increased irritability of the audience; I would like to point out to you that all of you have learned what many of you suspect--that at least part of your government will lie to you.

SESSION V. NERVOUS SYSTEM EFFECTS

INTRODUCTORY REMARKS
Morris Waxler

LIGHT AND ELECTRON MICROSCOPIC OBSERVATIONS ON THE BLOOD-BRAIN BARRIER AFTER MICROWAVE IRRADIATION
Ernest N. Albert

CALCIUM BINDING IN CEREBRAL TISSUE
Suzanne M. Bawin and W. Ross Adey

VESTIBULO-COCHLEAR SINGLE UNIT RESPONSES
Robert M. Lebovitz and Ronald L. Seaman

INTRODUCTORY REMARKS

Morris Waxler
Moderator
Division of Biological Effects
Bureau of Radiological Health, FDA

The nervous system offers unique opportunities for the specification of RF/microwave radiation hazards. Neuroanatomical, neurophysiological, neurochemical, and neuropsychological specificity has been approached for many sub-systems of the brain and spinal cord. In many instances, this specificity has clear implications for clinical neurology and clinical electroencephalography. Neuroscience was reared in the clinic with which it maintains close mutual sustenance. Marcel Kinsbourne's work in Toronto on the lateralization of language is intimately tied to the experiments of Sperry, Myers, Gazzaniga, and others on animals and humans. Have we looked for radiation effects on end-points related to this body of work? Karacan, Jouvet, Snyder, and others have demonstrated that reduction of REM sleep produces manic behavior in humans and animals. Have we looked for such changes? Hanrubia, Stark, Robinson, and others have demonstrated the clinical significance of the ocular motor circuitry. There are also numerous examples in the visual system: component analysis of the ERG, (Rodieck); spatial modulation transfer functions from the EEG of man and cat and its relation to amblyopia (Maffei and others).

On the other hand, there are many neural circuits, components, and constituents whose relationships to health and disease are little understood. Moreover, there are many animal behaviors whose empirical relationship to the animal's own body, let alone to the body of a human, is murky. We should examine each behavioral neuroanantomical, neurophysiological, neurochemical end-point which is being studied and ask whether obtaining an RF/microwave effect at any intensity relates to some specific human clinical problem; if it does not, where does it lead us?

LIGHT AND ELECTRON MICROSCOPIC OBSERVATIONS ON THE BLOOD-BRAIN BARRIER AFTER MICROWAVE IRRADIATION

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Medical Center
Washington, D.C.

INTRODUCTION

Microwaves have been reported to cause an assortment of physiological and morphological alterations in the central nervous system of experimental animals. These include tigrolysis and vacuolation of hypothalamic neurons (1,2), pyknosis of cerebral, deep cerebellar and brain stem neurons (3), nuclear eccentricity and karyolysis of spinal ganglia (1). The above mentioned alterations were reported after acute exposures (less than 1 hour) and at 20-100 mW/cm^2 power densities.

Similar biological effects have been obtained after low power density exposures (10 mW/cm^2 or less) of non-ionizing electromagnetic radiation (4,5,6,7). In addition to the above mentioned effects, electromagnetic radiation in the microwave range has been reported to cause hemorrhage, congestion, hyperemia and vascular edema (3,1). In our laboratory we have observed hyperemia and congestion of brain vessels but have not detected definitive evidence of hemorrhage post microwave irradiation (2). Recently Oscar and Hawkins (8) and Albert (9), reported increased uptake of mannitol in rat brains and extravasation of large protein molecules in the extracellular spaces in hamsters after microwave irradiation.

This study reports our current findings on the morphological effects of 2450 MHz microwaves at power density of 10 mW/cm^2 on the blood-brain barrier of Chinese hamsters.

MATERIALS AND METHODS

Chinese hamsters were irradiated with microwaves at 2450 MHz (cw) and power density of 10 mW/cm^2 for 2 and 8 hours for one day. Control animals were sham irradiated. All exposures were carried out at the Walter Reed Army Institute for Research located in Forest Glen, Maryland. Following irradiation the animals were anesthetized and treated for gross and microscopic (light and electron) examination as outlined in table 1.

During the evaluation stage, two individuals other than myself independently scored the brain slices for peroxidase leakage. Subsequent to embedding, semi-thin sections were cut and observed with dark field and bright field microscopy as well as electron microscopy.

Table 1

ANESTHETIZE animal with nembutal.

⬇

INJECT 10 mg Horse Radish Peroxidase in
0.2 cc saline via the femoral vein
& circulate 5 minutes.

⬇

PERFUSE using a modified Karnovsky fixative:
5 minutes 1% glutaraldehyde
5 minutes 4% glutaraldehyde

⬇

Leave in situ overnight.

⬇

DISSECT out the brain & rough trim; wash in
0.1 M Cacodylate buffer 45 minutes.

⬇

CHOP into 100 u sections on TC-2 Tissue
Chopper; rinse in buffer.

⬇

INCUBATE 45 minutes at R.T. on aggitator.
MEDIUM:5 mg 3-3' Diaminobenzidine in
10 ml Tris buffer (pH 7.6) & 0.1 ml of
1% H_2O_2 to yield a final concentration
of 0.01% H_2O_2.

⬇

Leave in buffer overnight.

⬇

EVALUATE sections & fine trim.

⬇

POST-FIX in 2% OsO_4 in cacodylate buffer.

⬇

EN BLOC STAIN (Sotelo).

⬇

DEHYDRATE in ethyl alcohol & EMBED for
light microscopy & transmission
electron microscopy.

RESULTS

Gross Observations

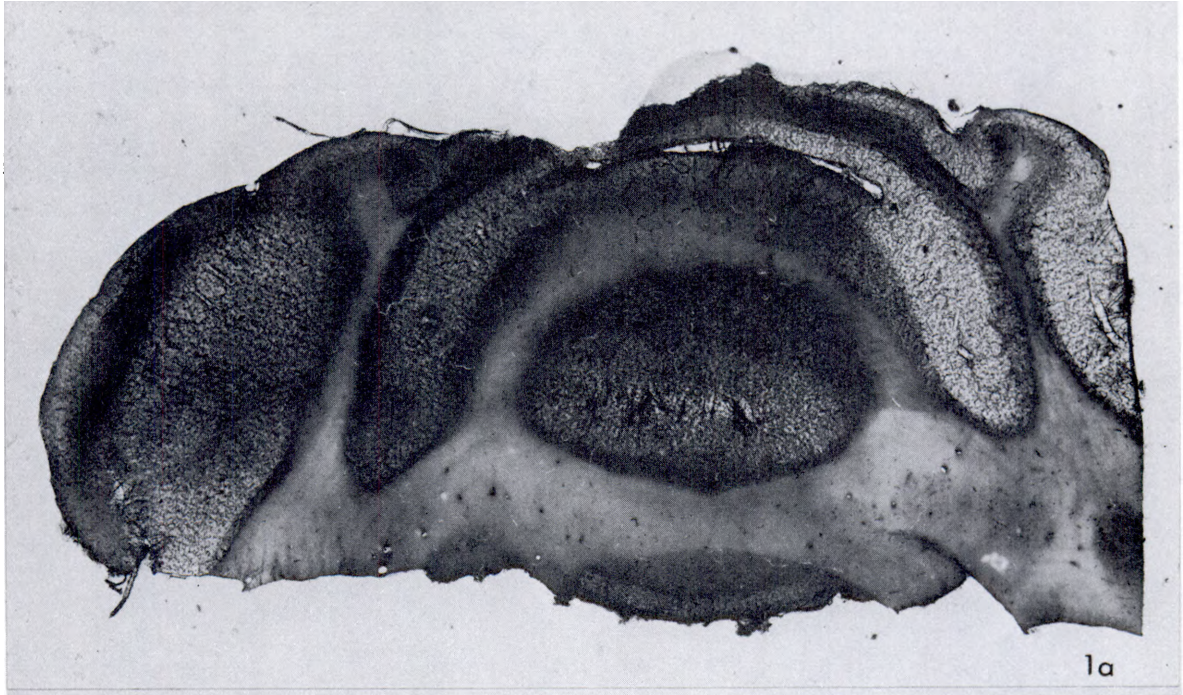
Horseradish peroxidase (HRP) incubated with 3-3 feet Daminobenzidine (DAB) in the presence of H_2O_2 yields a reddish brown reaction product. The distinct presence of this brown reaction product outside the brain blood vessels was used to identify areas of lesions or leaky brain microvasculature. Like brains of other animals, all brains of Chinese hamsters (control and experimentals) had some areas that displayed HRP reaction outside the blood vessels. These areas were the pineal gland, median eminence, area postrema, tuber cinerium, paraphysis, the wall of the optic recess and the eminentia saccularis. After the exclusion of these areas, it was noted that a distinct reaction product was, for the majority of cases, located in neuropil of experimental animals and absent in control animals. These lesions were scattered in random parts of the brains and showed no consistent pattern of localization in any functional region. One can clearly see the reaction product within the walls of intact blood vessels and contrast them with areas of leaky vessels (Fig. 1). This color reaction could also be seen in the white and gray matter. In addition, both controls and experimentals displayed some ambiguous pale brownish areas which were scored as mildly positive. Some of these areas were near the normally leaky regions while others were seen at distant sites from the special nonbarrier zones.

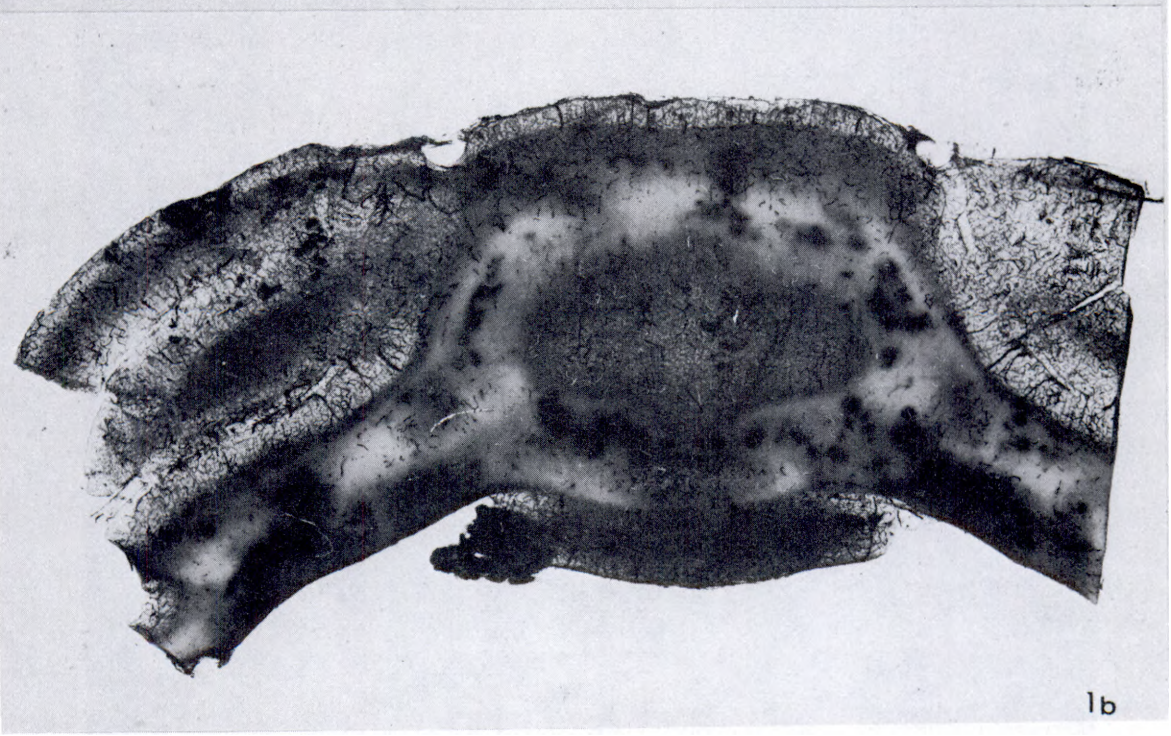
Light Microscopic Observations

Scanning of toluidine blue stained one micron epon sections from experimental animals revealed pale brown areas around some blood vessels. These areas were considered to be equivalent to leaky blood vessels evaluated as distinctly positive in gross sections; those areas that were categorized as mildly positive in the gross sections. The neuropil immediately surrounding the brown vessels often appeared edematous. These areas were considered to be the sites where extravasated HRP leaked into the surrounding neuropil. All other sections of experimental animals displayed colorless blood vessels, and the surrounding neuropil did not show any signs of swelling or edema. The presence of HRP in the neuropil was confirmed by dark field microscopy. HRP reaction product under these conditions appeared white and granular (Fig. 2) around blood vessels. It was most concentrated immediately around the vessels and became diffuse and sparse peripherally. The area of the neurohypophysis that is normally leaky was used as control for the darkfield evaluations.

Electron Microscopic Observations

Epon sections from the median eminence and posterior hypophysis (with fenestrated capillaries) and vessels with brownish peripheries from experimental animals were trimmed and thin sectioned. Electron microscopic examination from control and experimental animals revealed that electron dense HRP reaction product formed a thin layer on the luminal surface of the endothelium in most vessels. In addition, HRP was present in the basal lamina of normally leaky capillaries and venules of the neurohypophysis as well as in extracellular spaces surrounding these vessels (Fig. 3). Sometimes blood cells and HRP reaction product were present in the lumen of brain vasculature of all animals. This was interpreted to be due to incomplete perfusion of these vessels, but the fixation of the tissue was deemed satisfactory. Examination of leaky vessels from experimental animals revealed that HRP was present in the basal lamina, surrounding the pericytes and often could be traced from the basal lamina to the extracellular spaces (Fig. 4). In general, HRP-infiltrated areas of the neuropil exhibited swelling, edema and cytological damage. No reaction product





Figures 1a and 1b

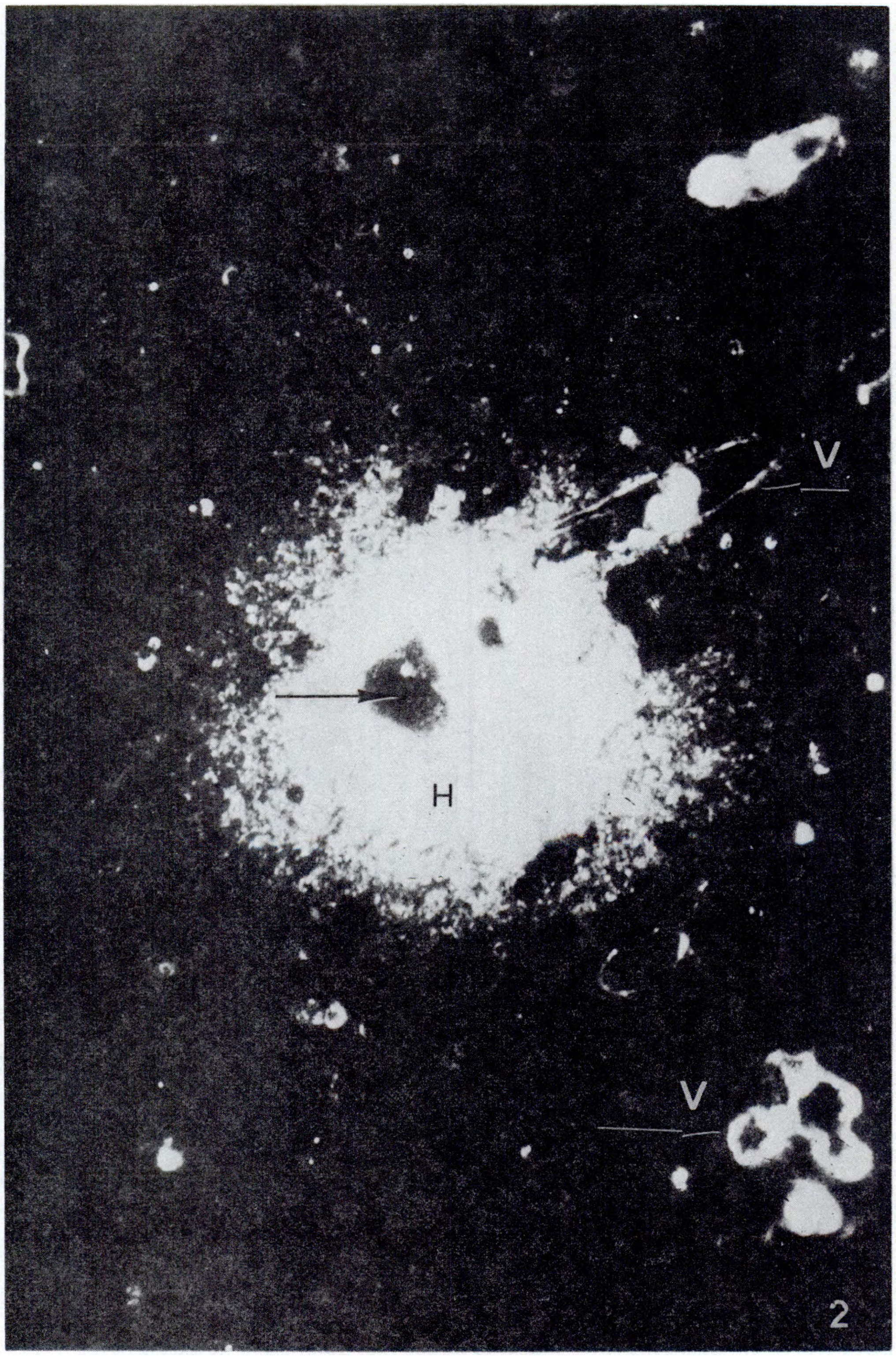


Figure 2

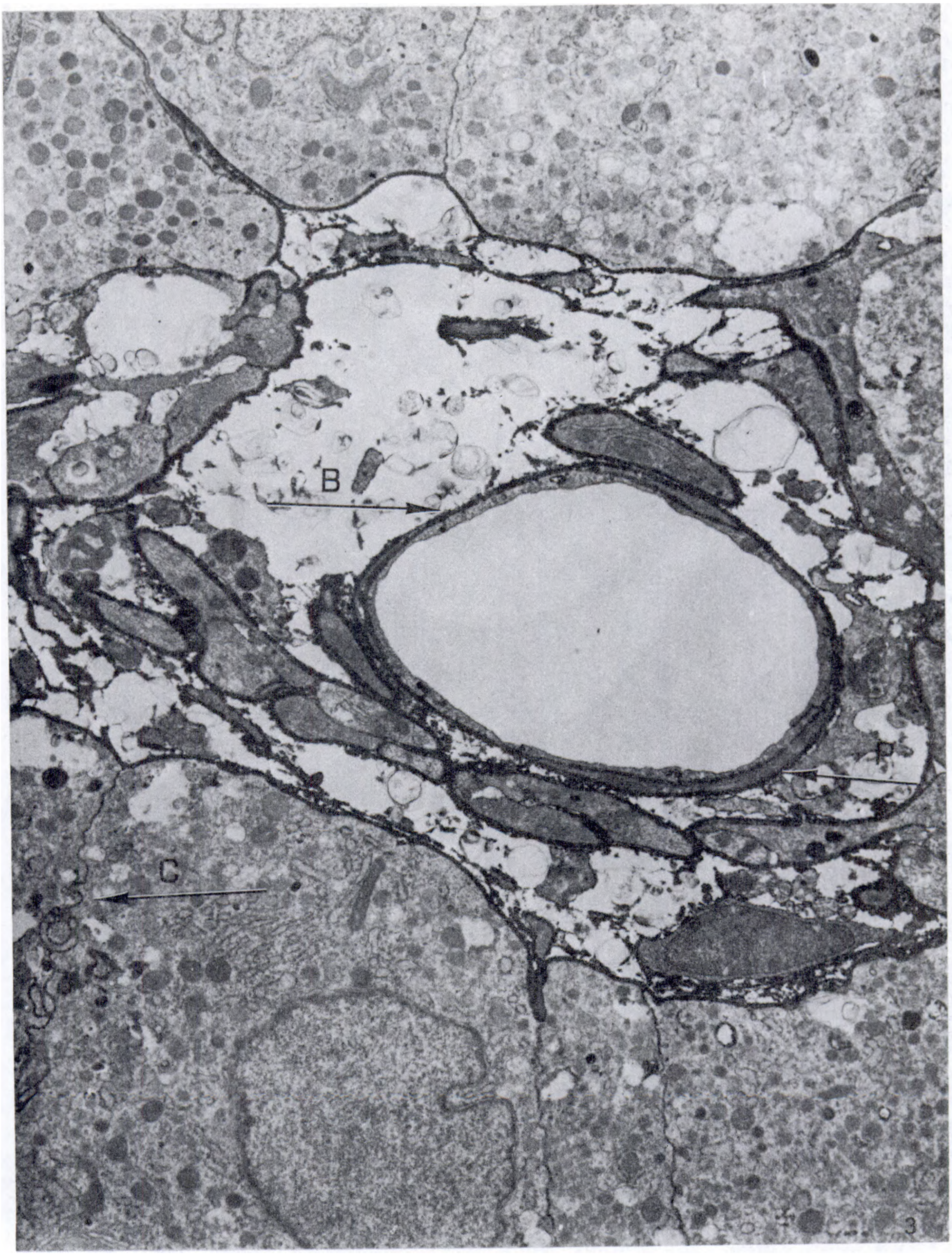


Figure 3

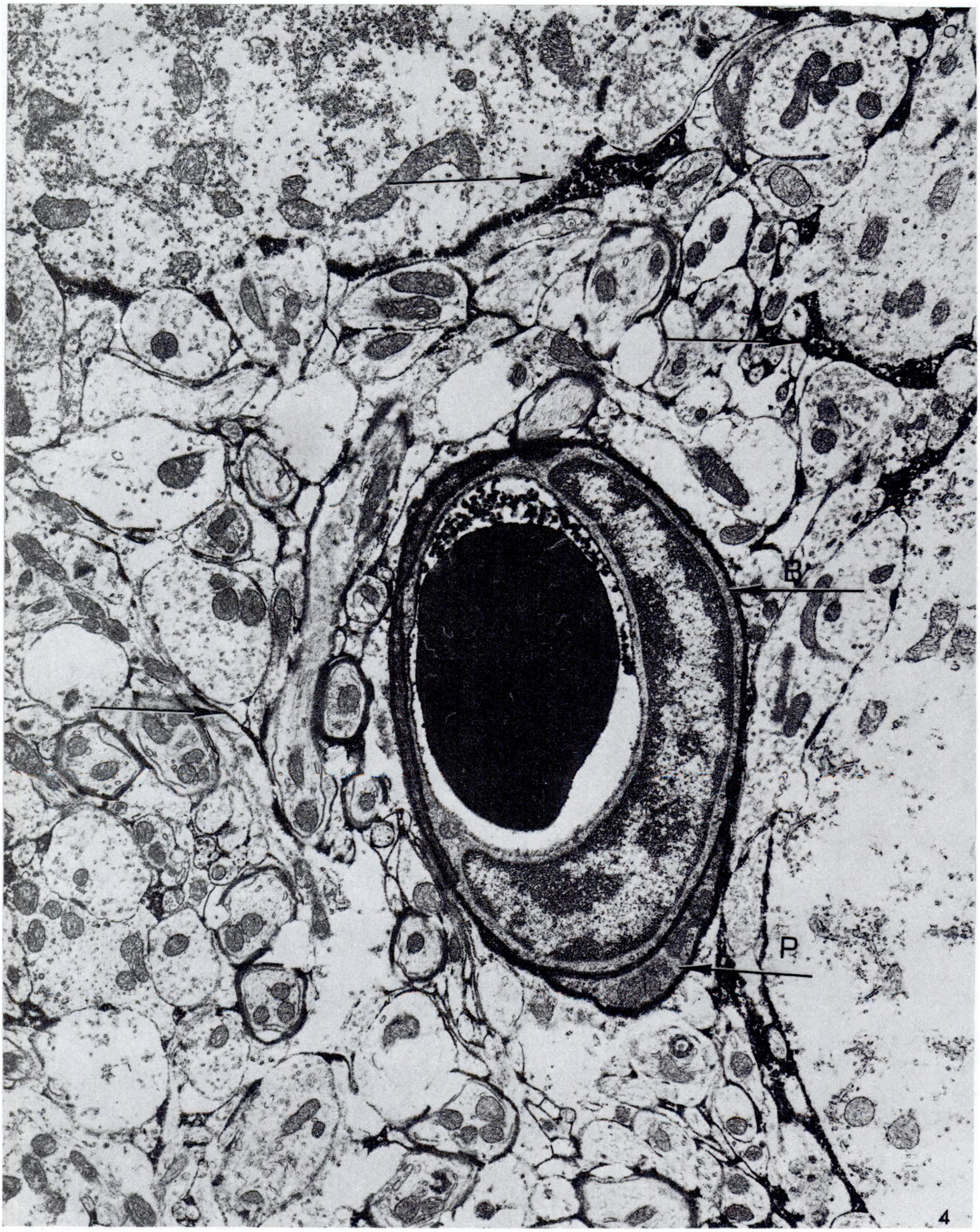


Figure 4

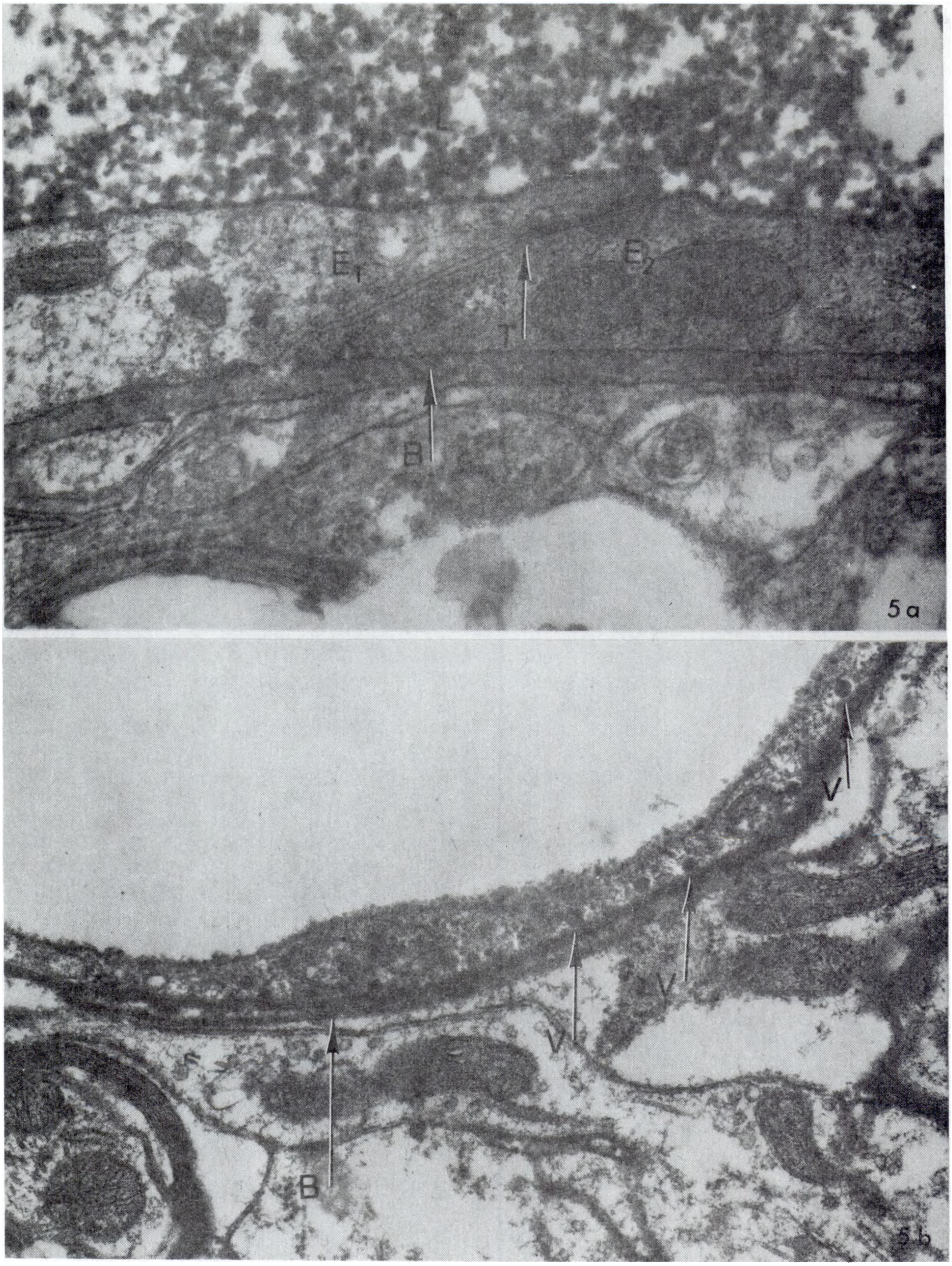
was observed in the neuronal and glial cytoplasm, dendrites, axons or their terminals. Blood vessels adjacent to the lesion areas were also examined. These vessels did not display HRP electron dense material in the basal lamina or in the surrounding neuropile. However, a fine endocapillary layer was often identified. Regions void of reaction product were interpreted to consist of nonleaky, intact blood vessels that were unaffected by microwave irradiation.

In an effort to determine the mode of exit of HRP from leaky capillaries to the basal lamina and the neuropil, the microvasculature was examined closely at higher magnifications. It appeared that the tight junctions between endothelial cells remained intact with little evidence of HRP passing through endothelial junctions. In most instances the electron dense reaction product entered only to the point where the tight junctions between endothelial cells started (Fig. 5a). Very few continuous lines of HRP were seen from the lumen to the basal lamina. In leaky vessels one could usually see pinocytotic vesicles filled with HRP and in close association with the basal lamina (Fig. 5b). In contrast the endothelial cells of nonleaky vessels from control and experimental animals demonstrated the presence of intact tight junctions and few HRP-loaded vesicles.

DISCUSSION AND CONCLUSIONS

It appears from the above observations that acute irradiation of Chinese hamsters with 2450 MHz microwaves at a power density of 10 mW/cm^2 causes alterations in the permeability of the blood-brain barrier to HRP in some vessels. The altered permeability of the microvasculature does not appear to be confined to any particular region of the brain. This conclusion applies only to molecules of 40,000 Molecular Weight or less (HRP Mol. Wt. 40,000). Larger molecules were not tested in this experiment. Whether this pattern will persist for longer or chronic exposures and/or other frequencies than tested in this experiment cannot be predicted at this time. These observations represent data obtained from animals showing a strong positive reaction. It does not include the blood vessels showing weak or pale reaction product. It should be pointed out that some blood vessels from control and experimental animals showed a weak positive reaction. The reason for this is not entirely clear at the present time. However, this weak reaction could be caused by endogenous peroxidase or some other factor. Evidence for endogenous peroxidase exists in the literature (Wong-Riley 1977). It is also possible that very small amounts of HRP normally leave the vascular walls in the brain but is insufficient to give a strong positive reaction. This is based on the observation that approximately 4 percent of plasma proteins are found in the cerebrospinal fluid.

Further, it can be stated that the exact mechanisms leading to the altered permeability are not clear either. It could be that the microwaves, which render it more permeable, have a direct and specific effect on the brain vasculature. It also could be that microwaves deposit energy in localized foci that in turn cause increased permeability via a heating effect. However, the thermal effect was not considered to be a significant factor in this study since the power density was 10 mW/cm^2 and the exposure was of short duration. Another area of great interest is the route of transport of HRP across the capillary wall to the basal lamina and the extracellular space of the neuropil. It has been reported that larger proteins such as HRP are restricted from entering the brain parenchyma as a result of the presence of tight junctions between endothelial cells (11,12). This barrier can be reversibly altered by opening the endothelial tight junctions through osmotic manipulation of blood (12,13). It also is commonly accepted that unlike capillaries in muscle tissues and other organs, brain capillaries have very few pinocytotic vesicles; therefore, vesicular transport has not appeared to contribute significantly to the overall movement of molecules. Observations described in this paper indicate little or


L
E1
E2
T
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5a
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V
V
B
5b

Figures 5a and 5b

no transport of HRP via interendothelial junctions. Further, it was observed that there were increased HRP filled pinocytic vesicles in endothelia of microwave irradiated animals. In addition some endothelial cells seemed to be filled with HRP. This could be due to toxic effects of HRP, but this phenomenon was not observed in control animals. Thus one could suggest that whatever the reason is for this cytoplasmic infiltration or toxic effect, microwaves may have rendered these cells more vulnerable to such agents. These observations suggest that transendothelial transport of large molecules may be initiated or enhanced by microwave irradiation. At the present time it is not the intention of this author to suggest exclusion of transjunctional movement of HRP in leaky vessels of experimental animals, but only to suggest that vesicular and other transcellular mechanisms may be the more prominent routes of moving large molecules in microwave stimulated brains. Further work on the blood-brain barrier and microwave effects is in progress and will be reported at a later time.

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OPEN DISCUSSION

MR. MONAHAN: You indicated that you had given repetitive exposures and that your acute exposures were of a 2-hour duration. I wonder if you could elaborate just a little bit on what your repetitive exposures consisted of and also if there were any observed differences between the acute and the repetitive animals.

DR. ALBERT: The exposures consisted of single 2-hour irradiation, 8-hour continuous exposure for 1 day, and 8-hour irradiation each day for 5 days.

In response to the second part of your question, it appears, and I want to emphasize the word appear, that repetitive exposures may show greater effects than single exposures. This is purely a subjective opinion and no quantitative analysis has been done yet. Today most of my discussion covered single 2-hour exposures. A few of my slides were from the single 8-hour exposure.

DR. MICHAELSON: Do you ever find the leakage of the peroxidase in control animals?

DR.ALBERT: Yes, we do. Normally, there is leakage of the peroxidase in about five or six areas of the brain because they normally are leaky. Second, surrounding these areas of the brain, it is very difficult to decide whether you are into an area or just past it. We have had a problem with that, but we have decided to score them as positives; and occasionally, we find animals that have leakage far removed from any of these areas. But again, explanations for that can be given because these molecules are not entirely stopped from passing. The idea is that there is normally about 0.5 percent of albumin found in the cerebral spinal fluid, but it is found in much larger quantities in the blood. There is, therefore, a movement of these large proteins, across the barrier under normal conditions. I do not think that our results are an all or none situation.

DR. MICHAELSON: That is an important point. Also, when you compare the irradiated animals to the control animals, are the control animals sham-exposed animals?

DR. ALBERT: Yes, they were kept with the experimental animals in every aspect, including being present in the radiation chambers, but they were shielded for the duration of the exposure.

DR. MICHAELSON: And they are shipped the same way?

DR. ALBERT: Absolutely. They come together in batches and are taken to Walter Reed and are kept there for 2 or 3 weeks before we start exposure.

CALCIUM BINDING IN CEREBRAL TISSUE

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ABSTRACT

We have investigated the possible effects of weak, extremely low frequency (ELF) and amplitude modulated radio frequency (RF) fields on the calcium exchange between freshly isolated nervous tissue and a bathing physiological solution. The experimental results indicate that cerebral tissue responds to field stimulations contained within amplitude and frequency windows. Isolated skeletal muscles do not respond to stimulations affecting the calcium exchanges in brain tissue.

INTRODUCTION

Inorganic cations are of extreme importance to living cells, as exemplified by the well known role of sodium and potassium in nervous excitability. Even though there is less calcium present in extra and intracellular fluids, the biological significance of the ion has been repeatedly stressed for many years and it is known that its function in the central nervous system alone ranges from control of structural integrity in cell membrane and intracellular organelles, to regulation of cell excitability and transaction of molecular events at the membrane surface.

The known properties of the calcium ion (Ca^{2+}) explain many of its unique biological interactions (1). Its hydrated salts exhibit flexible bond distances, bond angles and co-ordination number. Secondary interactions of its salts are determined by the dielectric of the medium, and includes hydrogen binding to water and other ligands. An example of this flexibility can be found in the ability of calcium to cross-link organic groups as seen in the neuronal membrane as well as in bone tissue, whereas no such cross-linking has been seen with magnesium ions which have a much more restricted range of structure.

Calcium is unevenly distributed between the cell and its environment. The calcium concentration in the extracellular compartment is of 2 to 3 mM, while the intracellular concentration is of the order of 3.10^{-5} mM. Studies of the squid axon (2,3) have shown that the intracellular free calcium concentration was as low as 0.1 μM, for a total intracellular concentration of 200-400 μM. Thus, most of the internal calcium is bound, partly to mitochondria, partly to cytoplasmic and membranous organelles and various substances such as ATP and glutamate.

The regulation of intracellular calcium sequestration and release is still being investigated. Even though mitochondria are certainly implicated in the sequestration of the ion, other buffers must also be involved since in axons fully poisoned by cyanide as little as 10 percent of the total calcium concentration is found unbound. A high affinity calcium buffer has been demonstrated in the squid axoplasm (4). Its concentration is of the order of 40 μM, and its affinity for the ion is such that it could effectively buffer against calcium variations in the range of tens of nM.

Calcium influx and efflux are to some extent mirror images of each other. The influx is a function of the external calcium concentration up to about 44 mM Ca. It is increased by depolarization of the membrane and by a reduction of the external sodium concentration (or an increase of the internal sodium); the efflux is a function of the intracellular calcium concentration up to about 100 nM levels. It is promoted by hyperpolarization of the membrane and by increase of the external sodium concentration. Mullins (2) suggested that the transmembrane calcium movements in the squid axon were controlled by a membrane pump energized by the sodium gradient across the membrane. The author calculated that in order to maintain an internal concentration of calcium of 30 nM against an external concentration of 3mM, a minimum of four sodium ions would have to enter the cell per calcium ion extruded.

We have studied the effects of weak oscillating electric fields on the binding properties of the calcium ion in isolated cortical tissue from cat and neonate chicks and in chick muscle (5,6,7). In order to avoid drastic ionic perturbations that would reflect on the ionic intracellular pools, we have adopted a balanced physiological solution with an average pH of 7.2, as an exchanger medium for cerebral tissues. Before being tested the samples are equilibrated for 30 min with the bathing solution, which contains 0.3 nM of radioactive calcium, and it can be assumed that transient calcium fluxes have been absorbed and that a relatively stable equilibrium exists in the tissue at the end of the incubation period.

The exchanges between the incubated tissues and fresh, non-radioactive solution were measured by liquid scintillation counting of aliquots from the bathing medium and of the digested tissues themselves, after 20 min of exposure to sinusoidal electric fields. Because the increment of energy in the system from the extremely weak environmental fields is almost negligible by comparison with the energies involved in transmembrane exchanges and because of the known higher sensitivites in transductive coupling at the membrane surface than for intracellular organelles, it is our belief that our experimental findings reflect the dynamics of calcium interactions with the membrane, rather than phenomena involving intracellularly bound calcium.

MATERIALS AND METHODS

Irradiations Chambers

Exposure to 147 MHz electric fields took place in a screened environmental chamber, kept at 37° C. Power supply radio frequency source and modulation function generator were located outside the shielded room. The coaxial feedline from the RF generator was applied via an antenna coupler to the narrow ends of two large aluminum plates. Identical voltages were applied to the plates with a 180° phase shift. The applied power, monitored by two in-line wattmeters was adjusted to provide field intensities of about 0.8 mW/cm^2. Sinusoidal low frequency signals (0.5 to 35 Hz) from the funtion generator were fed into a power supply which provided modulated high voltages to the transmitter. Modulation depth was maintained between 80 percent and 90 percent.

ELF field stimulation was carried out in the same chamber. Fields were established between two parallel rectangular metal plates, 50 cm apart. Equal voltages with respect to ground were applied to each plate. Sinusoidal electric fields at frequencies from 1 to 75 Hz and with electrical gradients in air ranging from 1 to 100 V/m were applied to the plates.

Exposure to the 450 MHz fields is taking palce in a TEM mode tapered chamber designed for irradiation of biological tissues and cultures, and of laboratory animals, including monkeys. The air conditioning system allows regulation of temperature (15 to 42 C) and humidity (ambient to 90 percent). The TE10 mode is excited in a section of a waveguide and drives a pyramidal horn. The horn flare is continued for 4.5 m and terminated by a large screened wall covered with microwave absorbing material. The 450 MHz generator is housed in a rack console together with modulation and power supplies. The crystal controlled RF oscillator is a solid state design with an output of 5.0 watts in a 50 Ohm load. The driver amplifier (output between 10 and 50 watts) and the final amplifier (output up to 500 watts) are screen-grid modulated. Sinusoidal or pulsed modulation of the carrier can be adjusted from 0 to 90 percent, between 5 and 35 Hz. The RF output is coupled to the waveguide through RG-8U coaxial cable and a discone probe. The space available for exposure situation at 3 meters from the throat of the horn, is about one cubic meter.

The chamber was evaluated by H.I. Bassen (**7**). Field distribution in the exposure space was found to be sufficiently uniform so that a quiet zone exists in the center of the space. Vertical polarization of the E field was found to minimize distortion, due for example to the introduction of a monkey in the exposure area. In this, volume, the field is uniform within narrow limits, and the standing wave ratio is less than 1.2:1 throughout the chamber.

Experimental Procedures

The methods applied in these experiments have been described in detail in previous publication (**5,6**). Chicks cerebral hemispheres and skeletal muscles were rapidly dissected following decapitation; cat cortical samples from visual, auditory, somatosensory and suprasylvain areas were painlessly dissected from immobilized, locally anesthetized animals. Brain and muscle samples were incubated for 30 min in 1.0 ml of physiological solution (155 mM NaCl, 5.6 mM KCl, 2.16 mM CaCl , 2.4 mM NaHCO and 2 g/l D-glucose), containing radioactive calcium (Ca^{+} ,0.2-0.3 Ci per ml, specific activity 18.5 Ci/g Ca). At the end of the incubation the samples were rinsed with non-radioactive solution before being tested, during 20 min epochs, for changes in the rate of ionic efflux during field exposure. Ten samples were tested at a time. Radio-activity was assayed by liquid scintillation counting.

147 MHZ Field Stimulation

Chicks cerebral hemispheres were exposed to 0.8 mW/cm fields, amplitude modulated at 0.5, 3, 6, 9, 11, 16, 20, 25 and 35 Hz, and to the unmodulated carrier. Controls were run in the absence of field. A minimum of 30 samples were studied under each condition. Thirty-five muscle samples were tested simultaneously with brain tissues in control experiments and at two frequencies of modulation (6 and 16 Hz).

ELF Field Stimulation

Chicks and cats cerebral tissues were exposed to fields at 1, 6, 16, and 32 Hz with electric gradients in air of 1, 5, 10, 56 and 100 V/m. Field exposures at 75 Hz were added in the cat brain studies. Fifty chick muscle samples were tested with 20 V/m, 16 Hz fields and compared with 30 non-exposed specimens. All samples were weighed before incubation to facilitate comparison between the effluxes obtained with cat and chick tissues, even though differences in shape and surface area of the samples could not be avoided.

450 MHz Field Stimulation

Chick brains were exposed to field amplitude modulated at 16 Hz, with intensities of 0.5, 1, 2, and 5 mW/cm . Considerable attention and time were devoted to optimizing the experimental procedures, especially the rinsing of tissue following incubation, which appeared to be one of the most critical steps in the experiment. In the latest and most satisfactory experiments, the 10 samples of a set are serially washed three times by immersion in fresh, non-radioactive physiological solution. The samples are then transferred to new glass test tubes, bathed in 1.0 ml of solution and exposed for 20 min to one of the experimental conditions.

RESULTS

147 MHz Fields

The release of preincubated $^{45}Ca^{2+}$ from the isolated chick forebrain was significantly increased, by comparison with the efflux from non-irradiated samples, following irradiation with fields amplitude modulated at frequencies between 9 and 20 Hz. Maximal increase (18 percent) was seen in the vicinity of 16 Hz. No effect was seen following exposure to unmodulated fields, nor with frequencies of modulation lower than 3 Hz and higher than 25 Hz. There was no difference between the Ca efflux from muscles exposed to 6 and 16 Hz modulation and control samples.

ELF Fields

This series of experiments confirmed the frequency sensitivity of the tissue response seen with the modulated RF fields. Significant decrease in the $^{45}Ca^{2+}$ from the chick and cat brain tissues was observed following exposure to fields of 10 and 56 V/m, oscillating at 6 and 16 Hz. Other frequencies tested (1, 32, 75 Hz) and other field amplitudes (1, 5 and 100 V/m) did not induce changes in the calcium efflux. Muscle tissue did not react to field stimulation (20 V/m, 16 Hz).

In addition to the frequency sensitivity, the brain tissue response appeared to be significant only in a field amplitude window between 10 V/m and less than 100 V/m. The mirrored images of the results obtained with RF and ELF fields will be discussed later.

Brain and muscle tissues, collected at the end of the experiments, were digested overnight in a solvent and assayed for radioactivity content. The ratio of $^{45}Ca^{2+}$ uptake versus release was about 3 to 1 for brain tissue and 1.5 to 1 in muscle samples. This point is important in establishing that an adequate pool of radioactive calcium was established in the brain tissue during the initial equilibration procedure.

450 MHz Fields

These studies are being continued and the results presented below should be considered as preliminary and therefore still incomplete. As mentioned earlier, we have tried to optimize the experimental procedures and alternated ways of washing the excess of $^{45}Ca^{2+}$ from the surface of the samples.

We have studied the rate of incorporation of calcium in the chick forebrain, in conjunctionn with the search for optimal experimental procedures. Brains incubated for 10, 15, 20 and 30 min were dissolved for radioactivity assays. The resulting counts, expressed in counts per min per gram (CPM/g) are shown in Table 1. It can be seen that the influx stabilizes after the first 10 min of incubation. The dispersion of the data decreased with time since the ratio of the standard deviations to the corresponding means decreased from around 0.2 after 10 min to 0.12 following 30 min of incubation.

Table 1. $^{45}Ca^{2+}$ Incorporation in the chick forebrain

Incubation time	N	m ± SD (CPM/g)
10 min	27	26,915 ± 6,027
15	26	31,806 ± 5,996
20	26	36,092 ± 4,225
30	25	45,732 ± 5,129

The mean radioactivity in each condition, given plus or minus the standard deviation (m ± SD) is expressed in CPM/g. N is the number of samples tested in each condition.

Irradiating the tissues with 16 Hz, 2 mW/cm^2 fields did not alter the rate of incorporation of $45Ca^{2+}$. Further experiments (see below) suggest an upper threshold in intensity for the field stimulation to be effective. Consequently the incorporation results obtained with 2 mW/cm^2 fields remain inconclusive at this time.

Twenty min exposures to 1 mW/cm^2, 16 Hz fields resulted in a marked increase in the release of preincubated calcium by comparison with control samples (9 - 10 percent, 70 paired samples, $p < 0.0005$). By contrast, 2 mW/cm^2 fields failed to induce measurable changes in efflux (1 percent. 63 paired samples, non-significant). Two other experiments conducted with 16 Hz fields at a higher intensity (5 mW/cm^2) confirmed the previous results, while three exposures of 10 samples to 0.5 mW/cm^2 fields (16 Hz) produced a major increase in the efflux. This experiment must be repeated however in order to be compared with the other extensively studied field conditions.

DISCUSSION

The experiments reviewed here have involved more than 1500 neonatal chicks and about 50 cats. The whole investigation has been focused on a single, basic phenomenon: the exchange of calcium ions between freshly isolated nervous tissue and a bathing physiological solution.

It would be misleading, however, to minimize the difficulties encountered in the experiment. Because the rate of diffusion of calcium ions in cerebral tissue is of the order of 1 mm/hr, we are obviously observing exchanges that take place near the cortical surface, which is easily damaged during dissection and further manipulation of the samples. Furthermore the rinsing of the tissue is of critical importance since insufficient washing increases the variability of the radioactive counts but prolonged rinsing results in excessive calcium exchange near the surface.

In our experiments, isolated cerebral tissue responded only to stimulations contained within the limits of "windows," in this case a frequency bandwidth (6 to 20 Hz) and amplitude thresholds (5 V/m < effective field intensity < 100 V/m, for ELF fields and an upper threshold at 2 mmW/cm^2 for RF exposures). The ELF and RF field effects were of comparable size, in the same low frequency range (frequency of amplitude modulation in RF), but of opposite sign. The mechanism and regulation of calcium binding at the cell membrane are still largely unknown, consequently only tentative explanations can be offered for the behavior of isolated cortical tissue.

No ready explanation can be offered for the frequency selectivity of the tissue response. Adey (9) has suggested a possible functional role for weak extracellular electric gradients in the maintenance and regulation of central nervous system excitability. The fact that only some frequencies in the EEG range, contained in a narrow window, were capable of eliciting a response, could be indicative of such an extracellular regulatory mechanism, functioning at extremely low frequencies. However higher frequencies as 250-500 Hz have not yet been investigated and no final conclusion can be drawn at this point.

We have suggested (6) that the observed increase and decrease in the $^{45}Ca^{2+}$ efflux could reflect the binding properties of calcium, and that different field stimulations (ELF versus amplitude modulated RF fields) could affect different binding sites. Calcium binds to oxyanions with a binding strength that can vary over several numbers of magnitude, and is involved in complex cross-linking and other cooperative interactions. Katchalsky (10) has studied the binding of the ion to a biopolymer sheet and demonstrated primary strong bonding at sites along a single strand as well as secondary, much weaker binding betweeen adjacent strands.

Given the essential validity of membrane surface models of the Singer and Nicolson type (11), we may anticipate first, that calcium ions could bind to cell surface glycoproteins, and that this binding may be influenced by extracellular electric gradients. Differential sensitivities would be expected at sites on terminal carboxylic groups, shared perhaps by the dual bonds of a single calcium ion in comparison with the weak "ladder formation" suggested by Katchalsky as occurring between adjacent strands.

It is in the magnitude of threshold gradients for these effects that our knowledge is weakest. We cannot postulate a priori on physical or chemical grounds what levels would be expected. Rather, we must be guided by observed levels of sensitivity, even when these appear to be substantially below those associated with classical transmembrane excitatory processes. Given that the observed sensitivities are valid, we are faced with yet another step in the sequence of transductive couplings from weak surface triggers to transmembrane excitation.

ACKNOWLEDGMENT

This investigation was supported by the Bureau of Radiological Health Grant USPHS 1R01 FD678, by the Naval Electronic Systems Command under an Office of Naval Research Contract N00014-69-A-0200-4037 and by the Air Force Office of Scientific Research Contract AF F-44620-75-C-0030.

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OPEN DISCUSSION

DR. ROSENBAUM: I would like to point out that there are at least two other indications of a power window in work on the heart rate of the frog and the turtle; there have been reports of no effect, bradycardia, and tachycardia at different power levels. This may also be an indication that in some of these interactions there is a power window. In our own work in the Tenebrio, we also have seen the disappearance of effect at certain power levels; so, I think this means there could be something valid.

DR.BAWIN: Could you give me an indication of the power level you are referring to?

DR. ROSENBAUM: These were substantially higher power levels. In the Tenebrio work, our effect seems to disappear in the vicinity of 80 mW/cm^2. However, I do not know your exposure mechanism or how to compare your numbers to my numbers.

DR. BAWIN: I also want to mention the work of Oscar and Hawkins at Walter Reed. They reported changes in the permeability of the blood brain barrier of rats irradiated with cw microwave fields up to 1 mW/cm^2 or with pulsed field at 0.1 mW/cm^2. This increase in the permeability of the blood brain barrier vanished at higher power.

MR. BASSEN: Is there anything in the literature of other physical agents affecting calcium efflux that would be of a similar nature?

DR. BAWIN: Yes. For example, experiments by Kaczmarek and Adey have shown that a local increment of the extracellular calcium concentration induced a local increase in the efflux of previously bound $^{45}Ca^{2+}$. Furthermore, the addition of 1 mM of calcium had practically the same effect than the addition of 25 mM of calcium, which is indicative of a highly nonlinear response.

MR. BASSEN: Would there be a possibility that thermal stress or other factors could cause this?

DR. BAWIN: Yes, certainly. Bystrom, Sabbot and Costin in our laboratory have shown that cold or heat stress increased the rate of calcium uptake in the cortex and pituitary gland of the rat. Mitochondria accumulated much more calcium following stress (4° C for 30 minutes or 32° C for 21 days) and were subsequently more resistant to mechanical injury.

MR. WAXLER: Do you have any plans to modulate in those particular band widths and to look at whether or not you have local effects on calcium efflux in those regions?

DR. BAWIN: Which band widths are you referring to? Higher frequencies?

MR. WAXLER: Either lower or higher. You are emphasizing, in particular, theta; how about some of the other frequencies?

DR. BAWIN: We have not seen any effect below 6 Hz or above 20 Hz in brain tissue. In studying muscle, we would like to use higher frequencies of stimulation--between 300 and 500 Hz--which would mimic the MEG more than the EEG.

MR. WAXLER: I think that would be a very important test.

DR. ALBERT: Do you have any idea what might be happening to potassium and magnesium ions at this time?

DR. BAWIN: No, I have no idea. I would like to study it, since as I said before, we are interested in ionic bonding in the CNS. For three years now we have been studying calcium alone, and it is not finished yet.

DR. ALBERT: So no data is being kept for future evaluation either?

DR. BAWIN: No, we would have to do separate experiments.

DR. KANTOR: This is very interesting. When do you plan to study the transport mechanisms involved? Intuitively speaking, I have the feeling (and unfortunately I am being recorded) that the mass of the calcium might have a lot to do with your particular frequency. The mass--the inertia--of the calcium ion might be tied in with your frequency, but you have to look at the transfer mechanism involved. This is a very complicated problem because you actually have a typical plasma physics situation. When there is an interplay between the different molecules where something is dragging and leading something else, and there is a change of frequency; obviously the microns are being moved, and they might then drag the ions along. These are very difficult questions. It is beautiful data, and as the previous gentleman pointed out, if you could relate the peak frequencies of the magnesium and potassium ions, then maybe you could get a better handle on whether this moves as a fluid or whether the particles move separately. I realize that these are very complex questions and, of course, I am not in your area. But from my previous experience in plasma physics, I can see the difficulty that you might have in interpreting it. However, since you have these very clean peaks in the frequency spectrum, you might have some very useful and theorical data that could lead you to simplify the transport equations, which can become very complicated. With your approach you might be able to cut out some of the parameters and come up with some very neat answers.

DR. BAWIN: Yes, perhaps; we certainly hope so. We are interested in other ions and their transport mechanisms; we just have not had the time to investigate them yet.

MR. SWICORD: It seemed to me that the work of Oscar did not have a completely independent power window and frequency window, using your terminology, and that there was a shift in the frequency response with power. Did you see similar effects to this?

DR. BAWIN: Not yet.

DR. MICHAELSON: Did you use 60 Hz modulation, also?

DR. BAWIN: I used 60 Hz in another experiment that I did not present here. There was no effect at 60 Hz and no effect at 75 Hz either.

VESTIBULO-COCHLEAR SINGLE UNIT RESPONSES TO MICROWAVE RADIATION

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ABSTRACT

Interactions of microwave radiation (MWR) with the mammalian central nervous system have received little study on the single cell level. Following the development of procedures that allowed reliable study of single neuron firing patterns during exposure to MWR, the influence of such MWR on functional neuronal properties was examined. The rationale having been developed in prior theoretical work, the effects of continuous wave MWR on units of the vestibular system were studied. Although significant and novel responses were observed, the threshold for this effect appeared to be above the current standards for safe exposure, i.e., above a level for significant intracranial thermogenesis. The development of a new clinical tool for vestibular diagnostics is projected; however, the responses of single auditory units to pulse modulated MWR were studied using a similar preparation. Pulse parameters rather than average power density appeared to be the more proper independent variable for this effect and responses were observed at pulse energy densities of 4 μJ/g and lower. Overall, the response of a given single auditory unit to pulsed MWR was very similar to its response to traditional acoustic click stimuli.

INTRODUCTION

One of the critical issues to have emerged from the work on the biologic effects of microwave radiation (MWR) is that changes in behavior may be induced by low levels of incident radiation (**1,2,3**). As an informal definition, let us take "low levels of MWR" to be simply an abbreviated way of saying "levels of incident radiation from which are derived physiologically insignificant changes in temperature in the test animal," viz., average incident power densities on the order of 5-10 mW/cm^2 and below. The observation of such behavioral changes correlated with exposure to low-level MWR does not necessarily justify hypothesizing an athermal mechanism. Highly localized absorption of the MWR (**4,5**), peculiarities of sensory receptor systems with regard to their thermal sensitivity (**6,7**) and, in the case of pulsed MWR, acoustic cues (**8-12**) could provide the basis for such behavioral changes.

If there are to be behavioral effects derived from MWR, then the nervous system must somehow be influenced. However, this is not to say that direct (for example, nonthermal) interactions with neural elements must occur. We simply do not yet know the answer to this question. Athermal interactions have been proposed (**13**), but verification of their existence, or even plausible hypotheses for their origin, have not been forthcoming. The point to be kept in mind is that mechanisms of a basically classical thermal nature can have novel neurological effects. Spatial or temporal temperature gradients, for example, can activate sensitive neural structures by a variety of intervening mechansims (**14,15**). Regardless of such speculations, the effects of MWR on neural elements have been characterized to only a limited degree. It has been our approach to study the mechanisms of interactions of MWR with neural elements without hypothesizing unverified "direct" effects. For this work, the influence of acute (short duration) MWR on the discharge patterns of single nerve cells in sensory pathways has been examined.

Single unit recording during exposure to MWR, even at low-average power levels, presents numerous procedural pitfalls. On the other hand, comparative studies of particularly well-researched physiological systems can reveal much about the nature of the MWR stimulus as it appears to those neural elements. A series of such comparisons would greatly enhance our understanding of basic MWR-biologic interactions that may have relevance for the nervous system and behavior.

In this report we focus on MWR interactions that appear to be mediated via the eighth cranial nerve. In the first part, we consider the effects of continuous wave-MWR (CW-MWR) on single cells associated with the vestibular system. A hypothesis had been developed from which a sensitivity of the semicircular canal component of this system to low to moderate uses of MWR radiation could be projected, given optimum irradiation geometry. The hypothesis (**15**) was essentially that of a derived intravestibular thermal gradient, hence, convective or "caloric" vestibular activation that would be dependent only on the average MWR absorbed dose. In contrast, other laboratories have already demonstrated the existence of auditory phenomena derived from pulsed MWR (**8,9,10**). For this effect the pulse parameters and not average power density seem to be the critical factor. The second part of this paper reviews the results to date of our studies of the response of single auditory neurons to pulsed MWR.

METHODS

Surgical

Adult mongrel cats of 1.5 to 4.5 kg body weight were used in these studies. All surgical procedures were carried out under general anesthesia with sodium pentobarbital (35 mg/kg IP initial dose; supplemental IV doses as required) or with a mixture of urethane (1 g/kg) and alpha-chloralose (40 mg/kg). The postero-lateral aspect of the cerebellum was removed by aspiration so that the recording micropipette could be placed in the eighth nerve just proximal to the internal acoustic meatus. When required, the surgical field was filled with a 3 percent agar-saline gel to reduce local pulsation. The animal was maintained at a core (rectal) temperature of from 36° to 38° C. Changes in body temperature as a result of the irradiation schedule were rarely seen. All wound edges were infiltrated with lidocaine so as to obviate physical distress and insure a stable preparation.

Physiological Stimulus Presentation

1. Vestibular. The animal was mounted in a stereotaxic baseplate that incorporated a headholder constructed of plastic (Plexiglas and nylon). This baseplate was mounted on a horizontal axle so that the entire assembly (including animal, microelectrode carriers, microelectrode preamplifiers, and microwave applicator) could be subjected to sinusoidal, low-amplitude oscillation ("rocking") in the vertical plane. Single unit activity could thus be recorded while the animal was rocking or while the animal was being irradiated.

2. Acoustic. Acoustic clicks were applied via a voltage-pulse driven condenser earphone coupled to one hollow ear bar of the stereotaxic apparatus by a short length of plastic tube. Acoustic pulse widths of from 10 to 200 μsec were utilized, although 70 μsec was the nominal standard value. The output acoustic intensity versus driving voltage was determined separately for each earphone by means of a hollow chamber that fitted over the microphone of a General Radio sound level meter and thus referenced to 0.0002 dyne/cm^2 (dB SPL). In the acoustic experiments, the contralateral ear of the animal was stoppled by a solid ear piece pressed against the external auditory meatus, but was otherwise intact. The background acoustic noise in the laboratory environment varied from 40 to 50 dB (broadband) and thus no doubt presented a sizable source of acoustic masking. This does not, however, detract from the general significance of the findings reported here.

MWR Stimulus

Pulsed modulated MWR 915 MHz from a MCL model 15022/6050 source was applied via a waveguide applicator constructed from a design kindly provided by Dr. A.W. Guy, University of Washington. The applicator was located approximately 3 cm from the dorsolateral aspect of the cat's head. The nominal peak CW output power of this source was 70 watts and reflected power was on the order of 20 watts. In several preparations the average incident power density was estimated by means of a Narda field intensity probe. However, the primary measure of applied MWR was the specific absorbed dose rate (SAR) as a function of net applicator feed power. This was obtained using a caloric method similar to that described by McRee (**16**). Once the animal was dead, it was subjected to successive standard intervals of CW-MWR irradiation. After each, a low mass thermistor was thrust into the medullar region of the brain stem, and the net temperature increase caused by that MWR exposure duration determined. For exposure durations up to 60 seconds, the rate of temperature rise was linear with respect to exposure duration, permitting the approximation that this rate of temperature increase was proportional to the local absorbed power per unit mass. Variations in the absorbed power through the head cross section were no doubt quite marked in this preparation; therefore, such determinations of midline power density should be taken as a reference value only. More detailed studies of intracranial MWR absorption profiles have appeared elsewhere (**17**).

All experiments were carried out within a shielded enclosure that was lined on three sides with microwave absorber (Eccosorb). The probe assembly of a Grass P-16 microelectrode preamplifier was enclosed within a second MWR shield and further protected by a low-pass input filter to reduce electrical artifacts resulting from each MWR pulse. These precautions enabled us to observe the effects of CW and pulsed MWR on single units in a stable fashion for extended periods of time.

Electrophysiological Methods and Data Analysis

Extracellular unit activity was recorded via glass micropipettes filled with 1-2 M NaCl. Each action potential or neuronal "spike" was converted to a 5 volt logic pulse via a window discriminator, and this logic pulse train provided the necessary information for analysis in terms of the distribution of interspike intervals. The waveform of each detected unit was monitored continuously via one trace of the oscilloscope display to insure reliable single unit discrimination.

Interval histograms and post-stimulus time histograms (PSTH) of unitary discharge were derived on-line via Ortec model 4621/4620 histogram and memory units. Given here in terms of counts per bin interval, these histograms are equivalent to the distribution of relative firing frequency, viz., firing frequency = (counts per bin) x (bin width)$^{-1}$ x (trials per histogram)$^{-1}$.

RESULTS

Absorbed Power Density

Since we were working with an "applicator" type MWR antenna placed quite close to the head, it was questionable that an estimate of irradiated power density, as by means of a field intensity probe, would adequately represent the level of radiation actually imparted to the test animal. Removing the animal from the stereotaxic frame and placing such a probe in an equivalent position introduces obvious uncertainties as a result of the unknown coupling characteristics of the head. Granted, if one were considering solely the risks or hazards of MWR, then a measure of incident power density would seem to provide the most readily transferable data. These topics already have received considerable discussion (**18**). In balance, however, when the object of study is a biological "effect" and, in particular, when different animal species will be used to fully explore the interaction, a measurement of the power actually absorbed at some constant reference point within the animal appears to be the most appropriate referent.

After the test animal was sacrificed, a thermistor probe plunged into the brain stem (at the midline, approximately at the level of entry of the eighth nerve) showed the expected slow but steady fall in temperature. After this regional temperature had fallen to 30° C or below, a determination of specific absorbed power density was made, as described above. This determination was always made in terms of effective irradiated power, i.e., the difference between applicator forward and reflected power. The data from nine animals are summarized in figure 1.

There was a distinct correlation between SAR (at the midline) and body weight. Since not every animal was subject to absorbed power density measurements, this derived correlation provided the basis for an adequate estimate of the absorbed dose rate. In studies utilizing pulsed MWR, the absorbed pulse energy density was calculated from the pulse duration plus the observed forward and reflected pulse power in the antenna feedline.

Response of Vestibular Units to CW MWR

The exquisite sensitivity of the semicircular canal system to mechanical, angular torque on its fluid-filled core suggested a hypothesis that might be referred to as "MWR-caloric" vestibular stimulation (**15,19**). The geometry of this apparatus system is basically that of a thin canal of cylindrical cross-section intersecting the vestibule, that has a much larger volume. The entire structure is embedded in bone. A straightforward thermal analysis (**15**) suggested that, even if the rate of volume heat generation (i.e., of MWR power absorption) were uniform throughout the region of the labyrinth, a significant thermal gradient could arise. This gradient would be established because of the differing rates of heat loss in the thin canal versus the larger vestibule. This hypothesis was examined by studying the response of single vestibular units in the cat to brief exposure of CW-MWR.

The typical experimental protocol was as follows. The animal was continuously "rocked" as the microelectrode was remotely advanced via a hydraulic microdrive. When a unit was obtained, its firing pattern relative to angular position and to angular acceleration was classified by deriving a plot of relative firing frequency versus angular position (phase) at several rocking

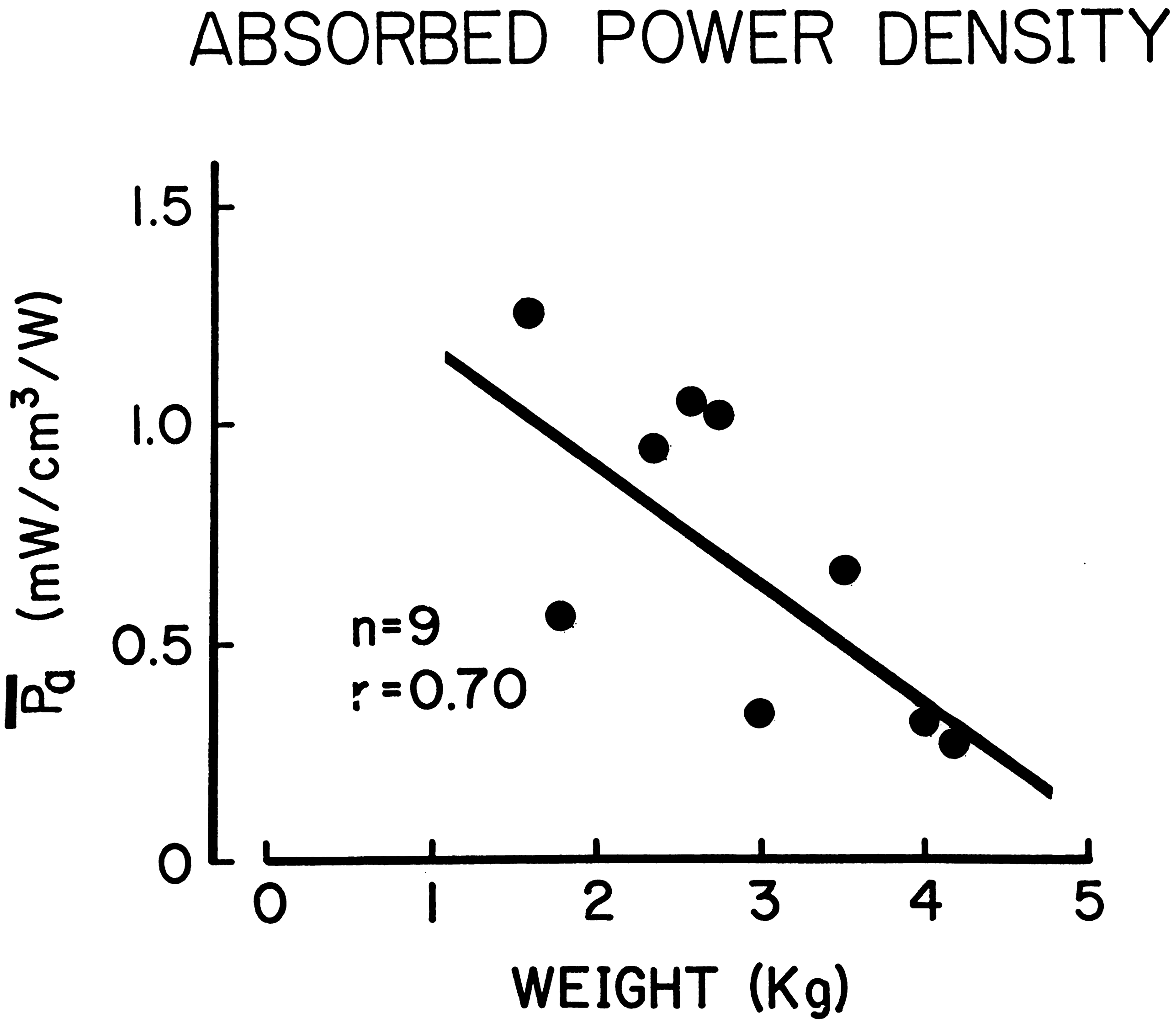

Figure 1. Midline specific absorbed power density (i.e., per watt effective irradiated power) as a function of animal body weight. A strong correlation is shown, which probably reflects the influence of head size and juxtacranial absorbed power density.

rates, as per the procedure of Kubo et al. (20). The relative phase was with respect to an initial position in which the head was in its extreme downward position. The animal was next fixed at some appropriate angular position and the MWR applied for 10-30 seconds over repeated 1-3 minute cycles, with exact timings and power level as dictated by the needs of a given experiment. The distribution of relative firing frequency over 8 to 32 such cycles was tabulated as a histogram utilizing a 0.5 or 1.0 second bin width.

Our classification of vestibular unit functional behavior was basically qualitative. In figure 2A are shown data from a unit classified as responsive to acceleration in the ventral to dorsal direction, i.e., head-up acceleration for the cat oriented in the prone position. Note that the peak firing rate increased with increasing angular acceleration, and also that maximal firing rate was at a relative phase of $\pi/2$, that is during the upstroke. As a result of an effective integration by the fluid within the canal, activation of acceleration sensitive vestibular units is in phase with angular velocity (20). The development of a weak MWR response is shown in figure 2B.

By way of comparison, figure 3A illustrates a unit whose response (firing rate) was maximal at a relative phase of π, i.e., head at maximum upward excursion. The peak firing rate of this unit also was independent of angular acceleration, and we would, therefore, functionally classify this as a position sensitive unit. The unit showed no response to CW-MWR (Fig. 3A), although the effect of different angular orientations of the head on the firing rate could be seen clearly.

Figure 4 similarly illustrates the response properties of a unit that was functionally classified as a downward acceleration unit. This downward acceleration unit showed a definite and dose-dependent response to repeated 10 sec CW-MWR exposures (Fig. 4B). The MWR threshold was no larger than 14.4 mW/g, in terms of midline absorbed power density. The slowing of this unit in response to the MWR was also dependent upon the duration of the exposure. For the 40-second exposure trials shown in figure 4C, note that the recovery after MWR exposure was considerably faster than the MWR induced slowing. Nearby, non-vestibular units showed no MWR response, as shown in figure 4D, a useful but not conclusive test for electrode-coupled artifacts.

A unit illustrating obvious position and not angular acceleration sensitivity is shown in figure 5. This unit showed no MWR response (Fig. 5B). For position sensitive units, the distinction between "head up" and "head down" was arbitrary in this format and was not made when tabulating the overall response properties of the 115 units examined in these experiments (see Table 1). The "questionable" category refers to units that showed a response but that were not held sufficiently long enough to complete all trials.

Table 1. Summary of brain stem single unit responses to CW-MWR

	MWR Response		
Vestibular Response	Responsive	Questionable	Non-Responsive
Acceleration, Upstroke	12 (35%)	4 (12%)	18 (53%)
Acceleration, Downstroke	4 (11%)	5 (14%)	27 (75%)
Position	10 (42%)	5 (21%)	9 (37%)
Mixed: Accel. + Position	3 (27%)	3 (27%)	5 (46%)
Non-Vestibular	1 (10%)	1 (10%)	8 (80%)

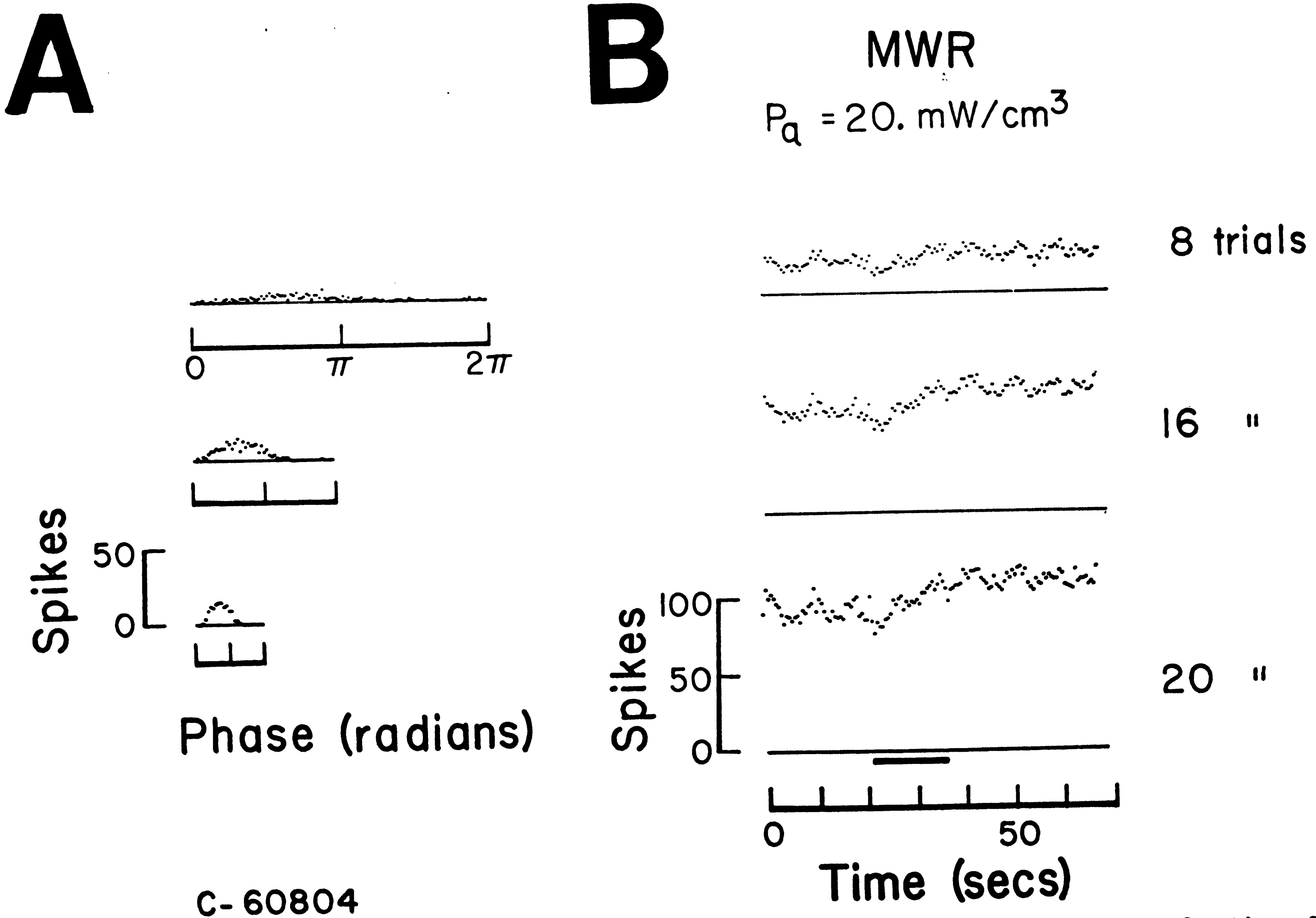

Figure 2. (A) Response histograms of acceleration sensitive unit. Relative firing frequency as a function of phase of rocking cycle (see text) is shown as the cumulative total spike counts per bin. Bin width was 50 msecs for each acceleration record. A standard format will be used here and in subsequent illustrations, with the rocking responses shown for three frequencies: from top to bottom, 0.18, 0.38, and 0.80 Hz. Maximum angular excursion was constant and these rocking rates represented peak angular accelerations of 4.7, 21.1, and 93.5°/sec^2, respectively. (B) Response histograms for repeated applications of MWR, applied during the interval demarked by the heavy bar. The level of MWR is indicated in terms of midline absorbed power density at the medulla. Again, the neural response is in terms of cumulative spike counts per bin (0.5 sec bin width) and the development of the response over repeated trials is shown.

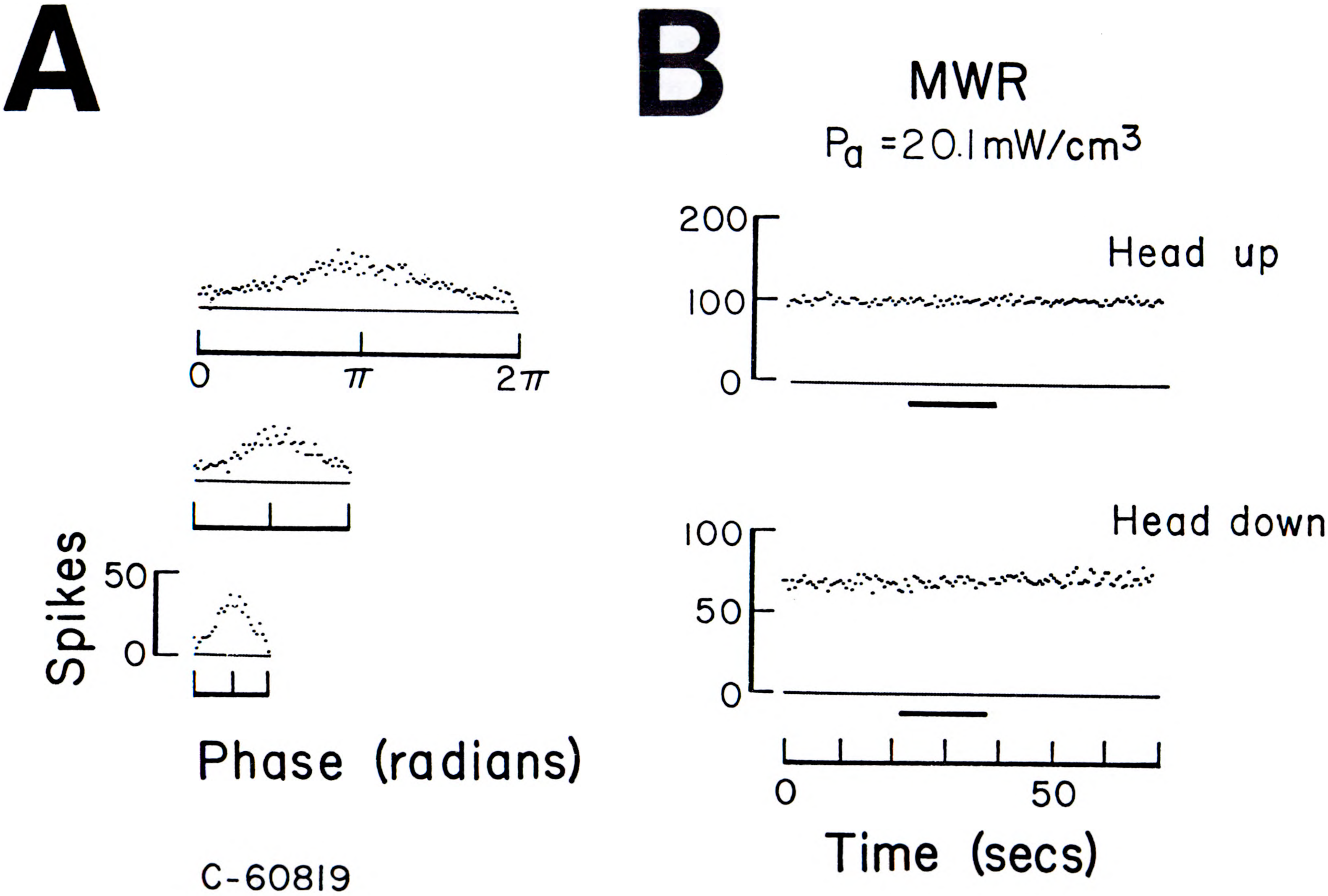

Figure 3. (A) Response histograms of a position sensitive unit. (B) MWR responses (null) at two different angular orientations. Eight consecutive applications at each position (0.5 sec bin width).

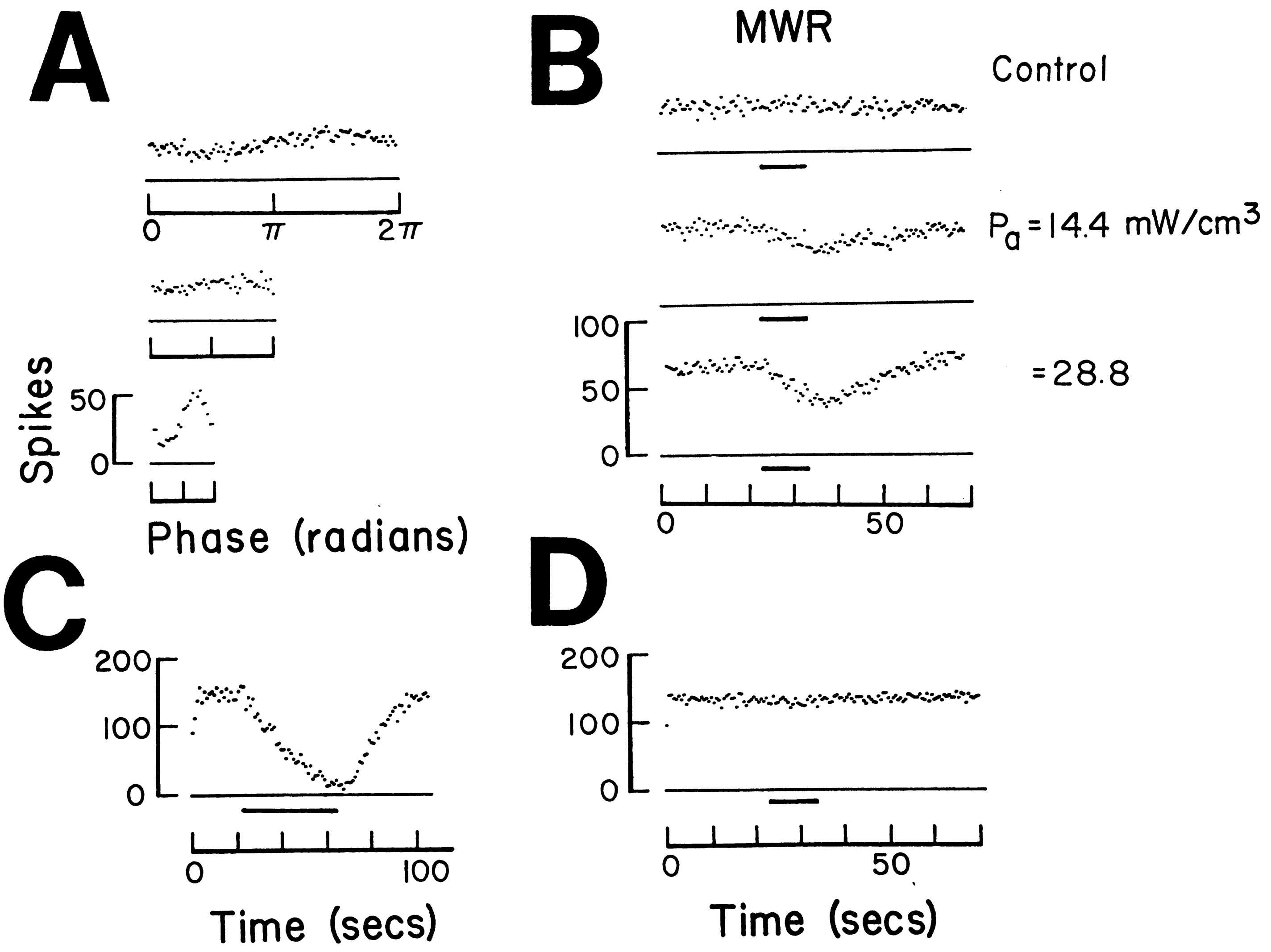

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Figure 4. (A) Response histograms of a unit response to downward acceleration. (B) MWR response to three levels of MWR, 8 trials at 0.5 sec bin width. (C) Same unit as in A, B, but MWR at P_a = 28.8 mW/cm^3 applied for 40 seconds, 8 trials at 1.0 sec bin width. (D) MWR response (null) at 28.8 mW/cm^2, from another, but non-vestibular cell in same cat and with same recording electrode.

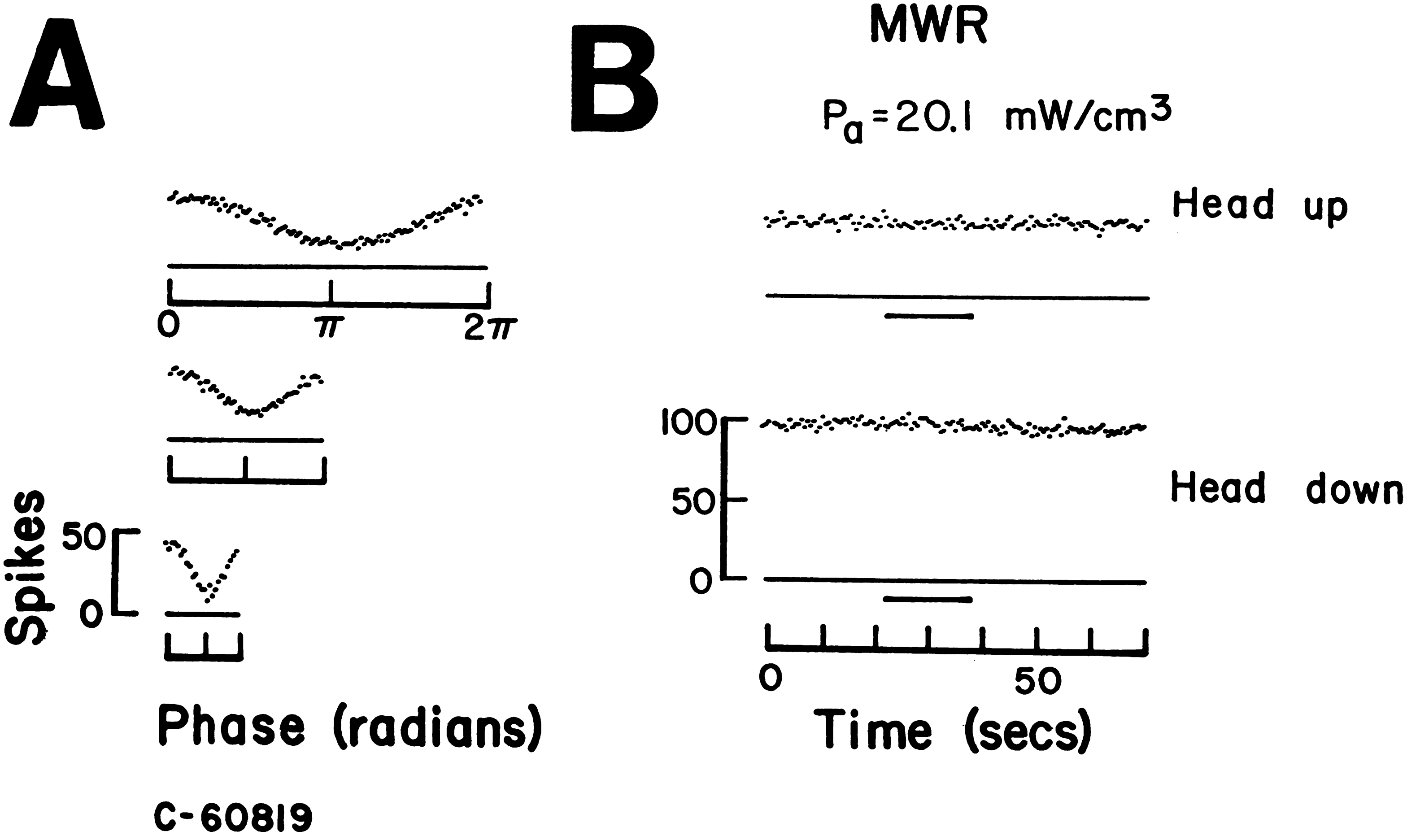

Figure 5. (A) Response histograms of a unit sensitive to position; maximum firing rate with head down. (B) MWR responses (null) at the two extremes of head position, eight consecutive trials each, and 0.5 sec bin width.

Evidence that the MWR responses observed in these studies was of vestibular origin and not incidental artifacts (local heating, coupling to electrode, ion leakage) were provided by unit pairs, as in figure 6. Here a unit showing a vestibular response (high threshold downward acceleration) showed a weak, dose dependent MWR response (Fig. 6A). Several hundred micra further along the same track, another clearly non-vestibular unit was held similarly for extensive study (Fig. 6B). No response to MWR could be observed. It was generally the case that MWR responsive and non-responsive units could be found with the same electrode.

This general impression, that the single unit response to CW-MWR was dependent upon the observed functional vestibular classification, is supported by the tabulation of data from the 115 units studied to date (Table I).

The total number of units classified as "non-vestibular" (ten) can be misleading since this represents actually those units which were fully tested for both vestibular response. Actually many additional units showing no vestibular response component were encountered. Cursory testing revealed no response from these to MWR in the 40 mW/cm^2 range. Although most records gave no evidence of an artifactual response induced by the MWR radiation, there was really no certain procedure by which to rule out localized neuronal heating. The essential point was whether any observed response to MWR could be directly linked to activation of the peripheral vestibular apparatus. The distribution of the positive results summarized in the Table 1 would tend to support such a contention, but is by no means conclusive. In only a few instances was the response to MWR as dramatic as in figures 4B and 4C. Whereas most (greater than 80 percent) of the units showing vestibular and MWR sensitivity gave evidence of an inhibition by the latter, the effect was generally weak, with apparent thresholds on the order of 20-30 mW/g in this format. Analysis of these data suggest that additional experiments using higher MWR absorbed dose rates are required.

Auditory Unit Responses to Pulsed MWR

The phenomenon known as "microwave hearing" has a long and variegated history. The original observations of Frey (**8**) appear substantiated in the main, and interest has been focused most recently on the mechanism, not existence of the effect. Since the acoustic response properties of single primary and secondary auditory fibers have been well worked out, these provide a means by which to assess the probable nature of the processes intervening between the absorption of incident MWR and the result and auditory effect. Since full reports have already appeared (**11,12**), here we will briefly summarize our findings with regard to microwave "hearing."

In each experiment, the animal was prepared as above. Again, localization of the recording electrode in the eighth nerve was accomplished under direct vision. Stable, extracellularly recorded single units were first evaluated with respect to their response to an acoustic click train by means of post-stimulus time histograms (PSTH) of at least 500 trials. The click rate was generally 10/sec or less. If still stable, a series of PSTH's with respect to MWR pulse trains (at the same repetition rate as in the acoustic trials) were obtained. If responsive to both the acoustic and MWR stimuli, a full sequence of acoustic and MWR dose/response determinations were derived.

Depending on the electrode characteristics and the state of the preparation, a MWR evoked potential generally could be detected. Its form was not grossly distinguishable from the acoustic click evoked potential, although the MWR response showed a smaller latency. This difference was on the order of

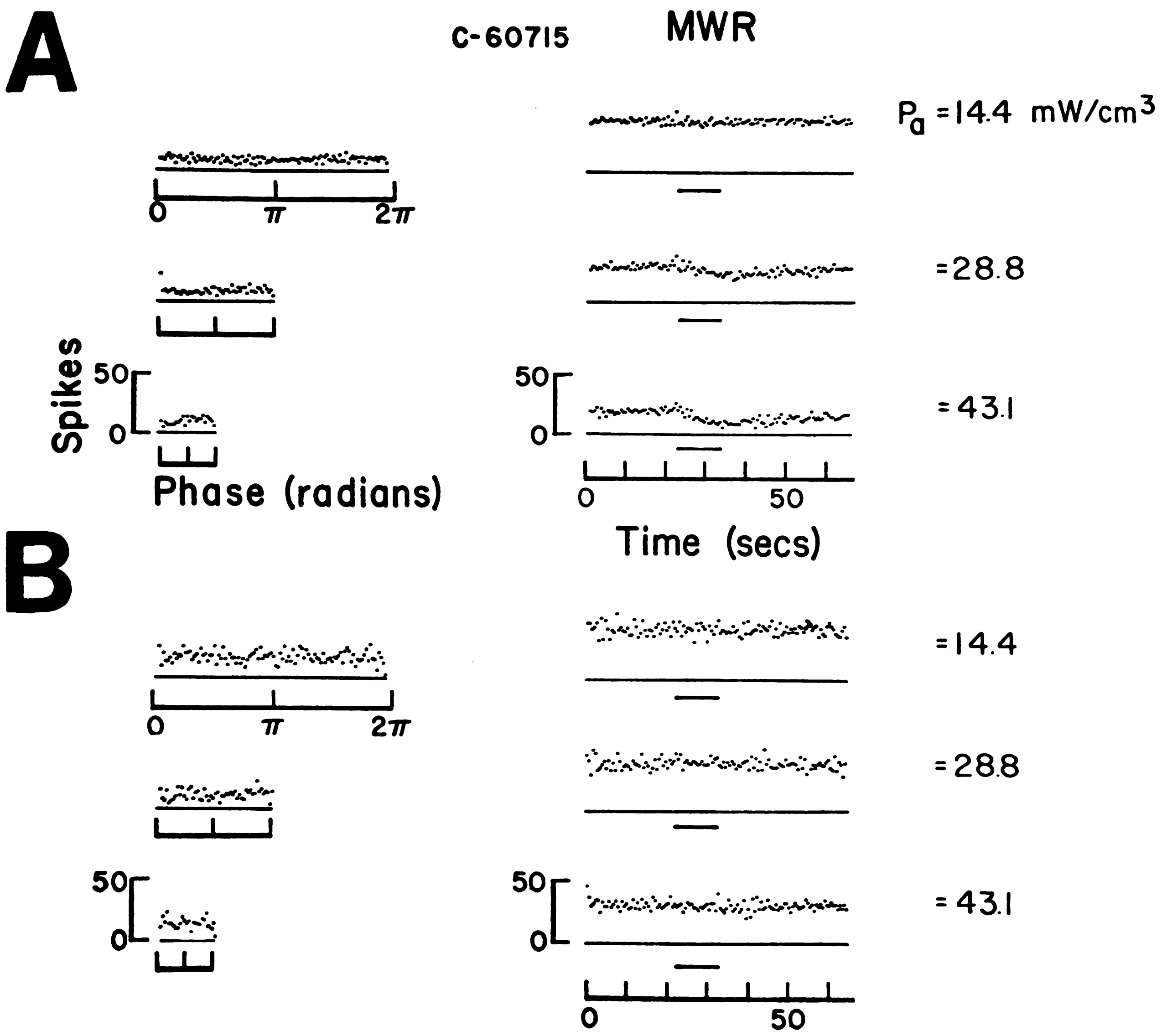

Figure 6. (A) Weak vestibular response to downward acceleration. (B) MWR responses at three increasing levels of irradiation (eight trials each, 0.5 sec bin width). (C) Another unit, several hundred micra further along same electrode track, showing no discernable response to physiological vestibular stimulations. (D) MWR response (null) of same unit as in C (null) to repeated MWR trials (eight trials at each MWR power level, 0.5 sec bin width).

0.5 to 1 msec. The magnitude of the MWR evoked potential was, however, highly dependent upon electrode location. The MWR evoked potential was frequently an early indication of whether our limited peak pulse power capability would be sufficient to elicit detectable single unit responses in a given animal. The maximum available pulse energy density was 40 µJ/g compared with a minimal detectable threshold on the order of 1-4 µJ/g. Thus, the effective range of the MWR pulse stimuli was much more limited than the 50-80 dB dynamic range of primary and secondary auditory units to acoustic stimuli (**21**).

Although the MWR-PSTH of a unit was frequently multi-peaked, the MWR stimulus intensity was seldom sufficient to yield multiple action potentials to a single pulse. Rather, the response took the form of a systematic modulation of the post-stimulus firing probability. The cumulative probability of discharge varied with changes in the MWR stimulus intensity (Fig. 7A) so that there appeared to be a general correspondence between MWR stimulus intensity and acoustic stimulus intensity. For pulse durations in the range of 25 to 300 µsecs, it was pulse duration and energy, not simply peak pulse intensity, that most strongly influenced the unitary response in most instances (Fig. 7A, B). As illustrated by that figure, equivalent pulse energies did not always yield equivalent PSTH's. Further, in some units, the relationship between the MWR stimulus intensity and unitary response was qualitatively more complex, as will be described in manuscripts now in preparation.

Figure 8 shows a series of MWR-PSTH's from a unit whose response was a non-monotonic function of pulse width as well as of pulse energy. In this unit, the maximal response occurred to MWR pulses of 150 µsec duration. No response was seen to MWR pulses greater than 250 µsec or less than 25 µsec in duration. In all of the units tested, it was found that at any given, fixed MWR pulse width, the response always decreased with decreasing pulse energy.

Since the response of each unit to PM-MWR showed distributions of post-stimulus firing intervals that were similar to their response to acoustic clicks (Fig. 9), it was feasible to estimate the approximate "acoustic equivalent" of the MWR stimuli on the basis of equivalent acoustic and MWR PSTH's. For a typical MWR-sensitive unit, for example, we observed that the response to a 10/sec train of MWR pulses of 250 µsec duration (corresponding to an absorbed energy density of 32.9 µJ/g per pulse) was comparable to that from a 10/sec acoustic click train at 60-70 dB SPL. This was on the basis of equal durations of the acoustic and MWR pulse stimuli, whereas an accurate specification of the apparent MWR sensitivity of each auditory unit would require consideration of each unit's acoustic frequency response.

The observations (1) that there was a considerable latency for the observed unitary response to the pulsed MWR, (2) that the MWR-PSTH was of the same form as the acoustic-PSTH, (3) that the single unit response could be a non-monotonic function of pulse width, and (4) that the same MWR-PSTH's were obtained whether the earphone remained or was removed prior to the MWR, confirmed that the MWR induced response did not in any way derive from electrical artifacts via the microelectrode or associated electronics. Short latency responses (under 1 msec) were rarely seen. Whereas such responses could have indicated a direct neural effect, it would be more likely that an electrical artifact was involved. Thus, non-propagated activity (i.e., originating in the vicinity of the electrode) was always excluded from these studies.

DISCUSSION

The recording of extracellular single unit data during either CW or pulse modulated MWR appears to be feasible and useful technique. No similar reports

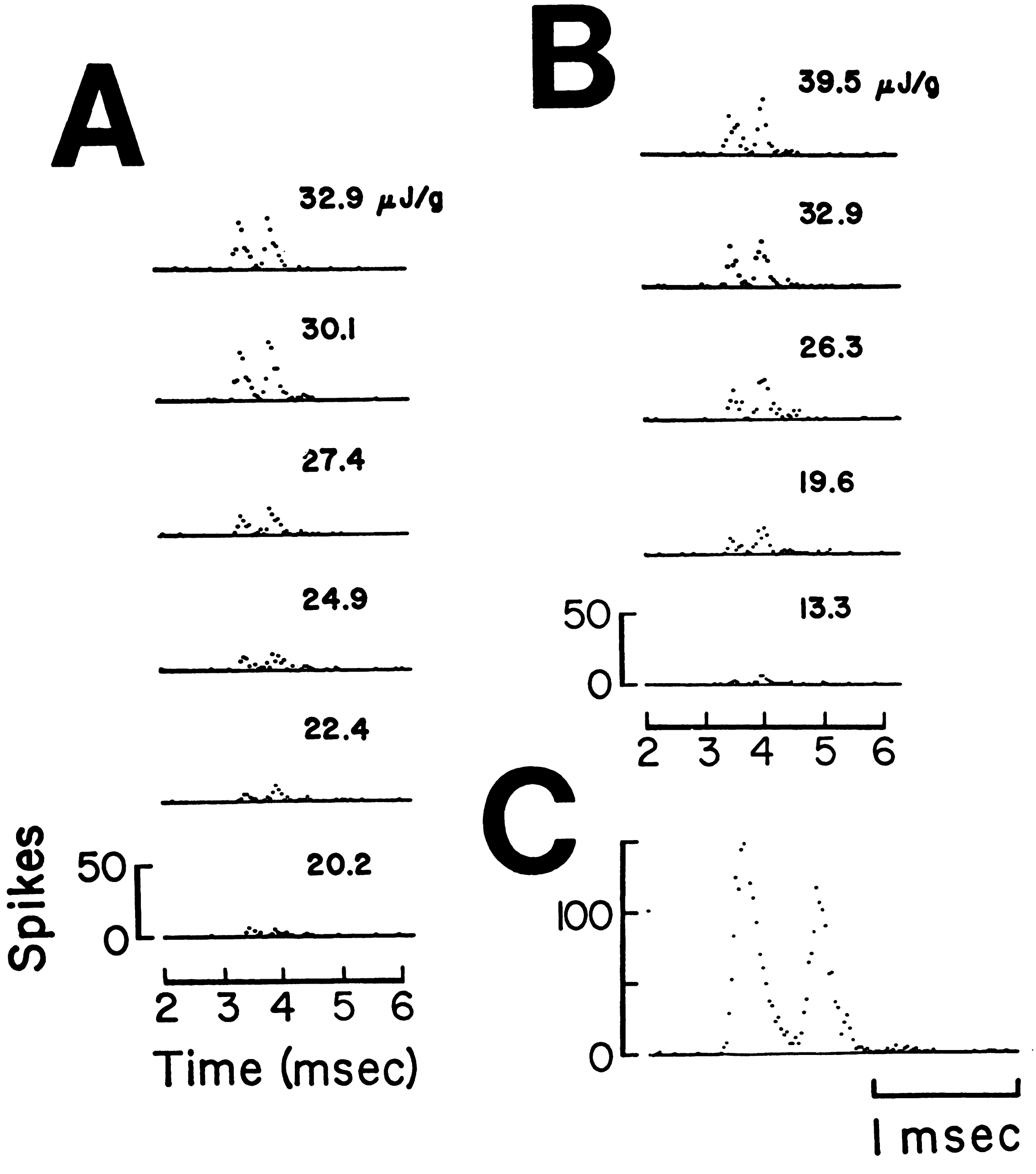

Figure 7. PSTH's of a unit for which the MWR response was dependent primarily upon pulse energy. (A) Responses to MWR pulses of fixed duration (250 μsec) and variable amplitude, as indicated by the absorbed energy density per pulse adjacent to each PSTH. Each PSTH represents 2,000 trials, at a bin width of 50 μsec. Time = 0 corresponds to the onset of the MWR pulse and in all subsequent MWR-PSTH's. (B) Responses of the same unit to MWR pulses of fixed amplitude and variable duration. Each PSTH again represents 2,000 trials, at a bin width of 50 μsec. From top to bottom, the pulse widths were 300, 250, 200, 150, and 100 μsecs, with absorbed energy densities per pulse as indicated. (C) The acoustic PSTH of the same unit shown above. Click rate was 10/sec, with a sound intensity level of 58.5 dB SPL. The PSTH represents 3,000 trials at a bin width of 20 μsecs.

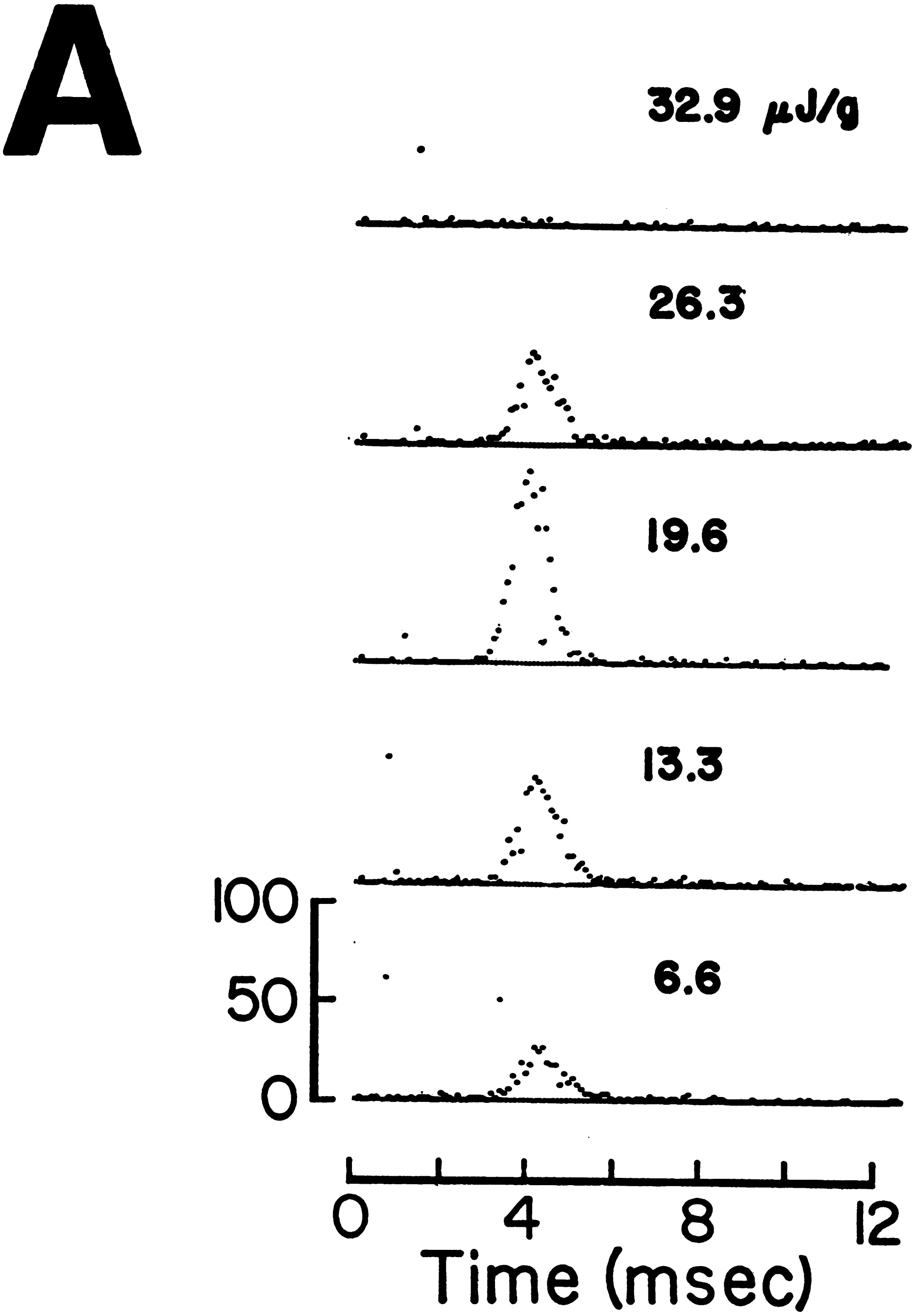

Figure 8. MWR-PSTH's of a unit whose response was a non-monotonic function of pulse width and absorbed energy density. The MWR pulse widths were, from top to bottom, 250, 200, 150, 100, and 50 μsec, with absorbed energy densities per pulse as indicated. Each PSTH represents 1,000 trials at 10/sec, with a bin width of 100 μsec.

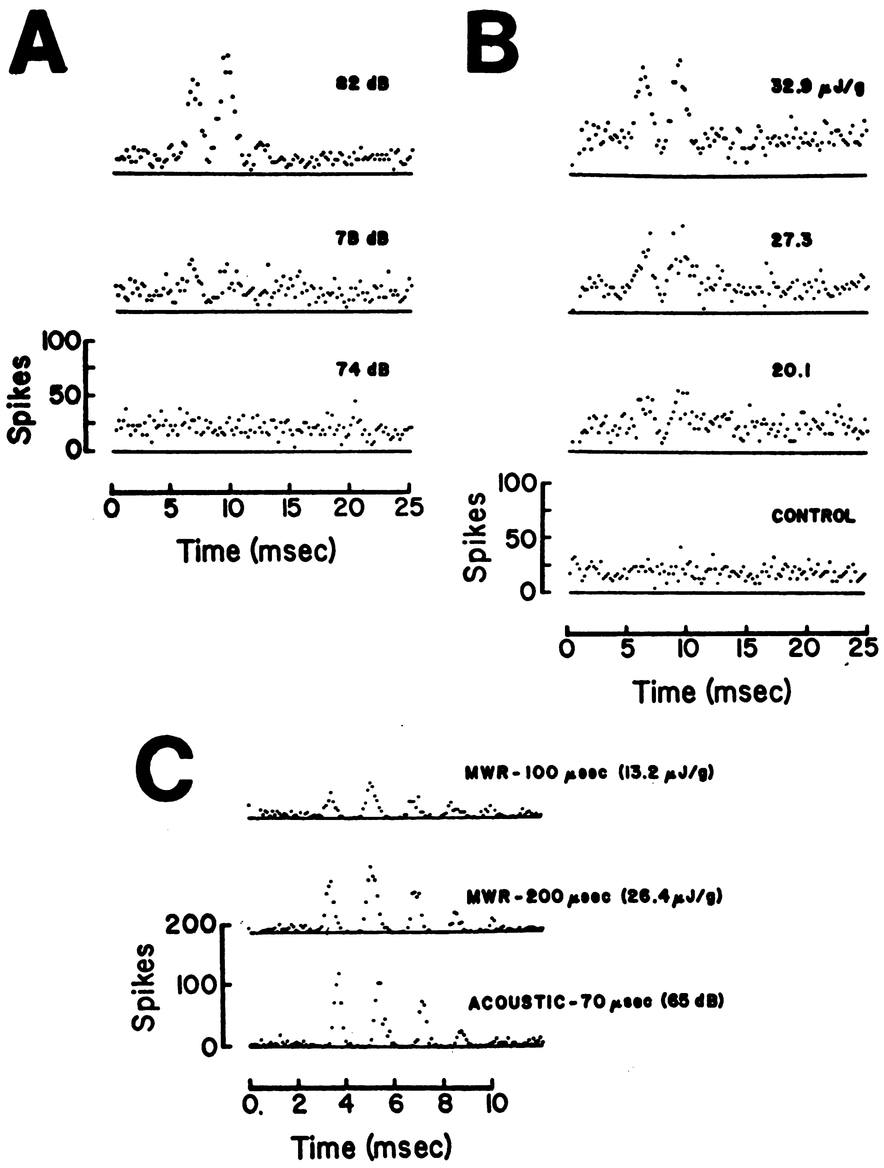

Figure 9. Comparison of the form and latency of acoustic and MWR-PSTH's. (A) Acoustic PSTH's at several sound intensity levels near threshold. A long duration (250 μsec) acoustic click was used, at a repetition rate of 10/sec, so as to be comparable with the MWR pulses used in B. The unit's acoustic threshold with respect to this click train (70 dB SPL) is, therefore, for reference only and not intended as an accurate representation of the acoustic sensitivity of the unit. Each PSTH represents 2,000 trials at a bin width of 200 μsec. (B) MWR-PSTH's from the same unit as in A; pulse width 250 μsecs with absorbed energy densities as indicated. Each PSTH represents 2,000 trials at a bin width of 200 μsec. (C) Comparable multipeaked PSTH's as obtained from another unit in response to both MWR and acoustic pulse trains. The stimulus parameters are indicated adjacent to each PSTH, which represent 500 trials at a bin width of 100 μsecs.

had appeared in the literature, and it was reasonable to expect that electrical artifacts or other technical difficulties would preclude or such studies. Our experience shows that the selection of high resistance NaCl-filled glass micropipettes and careful shielding of the preamplifier eliminates most such difficulties. A viable method for investigating the acute effects of exposure to MWR is now accessible. By concentrating first on sensory systems for which the patterns of neural activity have been well defined by prior work, some degree of confirmation of the physiological nature of the MWR effects could be obtained. That is, in our judgement, it is possible, to control for responses of a potentially artifactual nature.

The threshold for CW-MWR activation of vestibular units at 918 MHz, and using a diathermy type applicator, was significantly above current guidelines for "safe" exposure. The effect seems real enough, and it is with mixed positive and negative feelings that we note the threshold substantially agrees with that predicted earlier (15). On the one hand, it is both useful and encouraging to be able to support a theoretical hypothesis in the field of MWR research, where untested (or untestable) hypotheses can become quite popular. On the negative side, the utility in MWR activation of the vestibular system will depend upon developing means by which to improve the efficiency of the effect. However, in this regard there are several parameters that were far from optimum in these experiments--antenna configuration, antenna location, and MWR frequency. The development of a quantifiable clinical tool for the testing of vestibular function would be of considerable practical value and seems to be feasible on the basis of these data. These studies will, therefore, be enlarged and extended so as to explore the limits of the efficiency of the interaction.

It is highly significant that we have also been able to demonstrate directly for the first time the acute effect of pulsed MWR on single cells of the auditory system. The precise nature of this interaction is still open to question, although the range of possibilities is now somewhat reduced. It is clear that the interaction is with or within the head. For reasons that were outlined elsewhere (7,11,12) and from the form of the MWR-PSTH's, there is little likelihood that the responses described here arose from interactions of the MWR pulses directly with the cochlear nerve fibers, cell bodies at higher stations in the auditory system, the tympanic membrane.

The major utility of the data presented above lies not in the demonstration of single unit responses as such, but rather in what single unit activity can reveal about the locus and mode of interaction of the MWR stimulus. The most satisfactory hypothesis to date for explaining microwave hearing has been that the incident MWR pulse elicits a thermoacoustic wave in the head that then mechanically stimulates the inner ear via bone conduction (22). This would imply that the response of neurons in the auditory pathway to pulsed MWR would necessarily be similar to their response to transient mechanical stimuli, such as acoustic clicks. Unfortunately, our data do not distinguish between primary and secondary auditory units; however, it appears evident that the MWR-PSTH and acoustic-PSTH of a given MWR-responsive unit are indeed sufficiently similar to suggest an equivalence between the two stimuli at the auditory periphery. Further, there is evidence that the frequency content of the MWR pulse envelope (relative to the characteristic frequency of the unit) determines the magnitude of the MWR response (12). One could conceive of the PSTH's having grossly different forms in response to acoustic and MWR stimuli. Such differences would have supported the concept of a direct interaction with the hair cells of the cochlea, or perhaps with discrete portions of the basilar membrane. However, such unique responses were not reliably demonstrated and the data are most consistent with physiological activation of the auditory periphery via some mechanical mechanism.

Some difficulties with the current thermoacoustic hypothesis arise because of the decreased MWR sensitivity of high-frequency auditory units. The finding of a pronounced high frequency (10-30 KHz) cochlear microphonic (23) would imply that high-frequency auditory units in the cat would be most responsive to PM-MWR. Our data in this regard are admittedly incomplete; however, it is worth considering that this cochlear microphonic, though present, might be secondary to some other primary acoustic transient or that the envelope of this microphonic better represents the acoustic equivalent of the MWR pulse. On the other hand, our study has utilized broad MWR pulses (100 - 250 µsec or longer) that could induce mechanical transients having significant low-frequency content. Clearly, microwave hearing is more complex than as first presumed and an interaction of the MWR directly with some intracochlear component cannot yet be totally ruled out.

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OPEN DISCUSSION

MR. BASSEN: You indicated absorbed power in the watts per cubic centimeter. How was this data obtained in the near field exposure?

DR. LEBOVITZ: This was done afterwards in cadaver animals with identical situations. The animal was irradiated for selected intervals of time, after which a thermistor was plunged down into the area of the medulla, right at the level of the nerve. We followed the fall in temperature and projected back from that to get the difference in temperature from the beginning to the end of the irradiation. These studies were done after the animal had expired. We would, then, go back and have calibration that was sufficient to determine what that animal actually received.

What was the temperature change induced on the vestibular apparatus in your experiment? I can tell you what kind of heating we got at maximum incident power density during the calibration runs. With maximum power for 1 minute of irradiation, we got less than a 1° change. I would estimate for the values shown in the figures, and for the 20-30 seconds of irradiation during the experiment that we would actually observe less than a 0.1° change.

DR. CARPENTER: Are you assuming the same temperature change in the parilymph and endolymph of the system?

DR. LEBOVITZ: In the analysis, we did. In the experiments, of course, we do not have the intravestibular thermal data. In the analysis, this is a very reasonable assumption since the membrane between the two is quite thin. However, the analysis (**15**) did take into account the thermal gradients across the canal diameter as well as along the canal axis in the circumferencial direction.

DR. CARPENTER: I must admit serious reservations after seeing your drawing of your Plexiglas holders. With that amount of Plexiglas in the field, I do not see how you have any idea of what you are giving the animal.

DR. LEBOVITZ: As I said, we measure it directly. We are measuring absorbed power density (SAR) afterwards at the brain stem using a caloric technique.

DR. CARPENTER: Thermally?

DR. LEBOVITZ: We irradiate the animal for an interval of time, measure the rate of temperature rise at the brain stem; and from that measure of irradiation for periods of 10, 20, 40, and 60 seconds, and from the rate of rise of temperature at that area, we calculate the absorbed power. As long as we are in the linear region of the temperature curve, this provides a satisfactory measure of regional absorbed power density.

DR. CARPENTER: I can conceive of your getting the same rate of rise, but not the irradiation following a completely different path as it bounces from one Plexiglas holder to another causing refraction.

DR. LEBOVITZ: I do not think I am making myself clear. The numbers that we put up are measured values. The animal is in the holder and at the conclusion of an experiment, we remove the microelectrode and then begin thermal measurements; so, we are actually measuring the rate of power absorption at the animal's brain stem.

DR. CARPENTER: Yes, that is probing for temperature.

DR. LEBOVITZ: Right.

DR. CARPENTER: You can get the same temperature rise with various incidences of microwave power applied to the area?

DR. LEBOVITZ: Right; that is why we are trying to get a measurement of the absorbed power.

DR. CARPENTER: In the brain stem?

DR. LEBOVITZ: At that area, so it is a highly regional measure. It is a near-field situation and, perhaps, the alternative would be to determine with a probe what the output of power density of the applicator was.

DR. CARPENTER: That would not do you much good.

DR. LEBOVITZ: No, it would not, and so we rely on this measure that tells us more of what is actually being absorbed during the experiment. The translation to instant power density is difficult in the situation; that is why we do not choose to report in that fashion.

SESSION VI. OCCUPATIONAL AND OCULAR EFFECTS

INTRODUCTORY REMARKS
Charlotte Silverman

CAUSES OF DEATH FOLLOWING OCCUPATIONAL EXPOSURE TO MICROWAVE RADIATION (RADAR) 1950-1974
C. Dennis Robinette and Charlotte Silverman

EFFECTS OF REPEATED MICROWAVE IRRADIATIONS TO THE ALBINO RABBIT EYE
S. E. Hirsch, B. Appleton, B. S. Fine, and P. K. Brown

HISTOPATHOLOGICAL CHANGES DURING DEVELOPMENT OF MICROWAVE CATARACTS
Russell Carpenter

ARE MICROWAVE CATARACTS THERMALLY CAUSED?
R. L. Carpenter, G. J. Hagan, and G. L. Donovan

HEAT-INVOKED CHANGES IN ASCORBIC ACID LEVELS IN THE RABBIT EYE
Esther S. Ferri

CONCLUDING REMARKS
Moris L. Shore

INTRODUCTORY REMARKS

Charlotte Silverman
Moderator
Deputy Director
Division of Biological Effects
Bureau of Radiological Health, FDA

Because there is only one study in the occupational area, and because it is a major study; a little background information may be important. In 1970-71, under an interagency research contract from the Office of Naval Research, the Bureau of Radiological Health did a pilot study to determine the requirements and the feasibility of a proposed epidemiologic study of health risks from microwave radiation. The study was to investigate the mortality and mobidity experience, reproductive performance, and health of the children of occupationally exposed and non-exposed enlisted men in the Navy. Pilot work was done in collaboration with Dr. Gilbert Beebe of the National Academy of Sciences and with the assistance of various Navy specialists. I presented a status report at the Electromagnetic Research Workshop of the Department of Defense at the Navy Bureau of Medicine and Surgery in Washington in January 1971. The pilot work showed that the proposed investigation could be done, but the cost of studying all the factors of interest would be very high. Considerable additional cost would result if the study were extended beyond military and veteran records to include follow-up questionnaires, review of civilian records, household interviews, and selected reexaminations.

With these considerations in mind, a study protocol was submitted to the Navy in 1971 involving only military and veteran records research. Unfortunately, funds were not available at the time even for the modified study. In 1974, the Bureau of Radiological Health was able to provide funds for several basic elements of the study and a contract was given to the National Academy of Sciences. We are very pleased that the 3 year study is now in its analytic phase.

CAUSES OF DEATH FOLLOWING OCCUPATIONAL EXPOSURE TO MICROWAVE RADIATION (RADAR) 1950-1974

C. Dennis Robinette
Medical Follow-up Agency
National Research Council
National Academy of Sciences

Charlotte Silverman
Division of Biological Effects
Bureau of Radiological Health, FDA

For the past two and one-half years the Medical Follow-up Agency of the National Academy of Sciences-National Research Council has been involved in a study whose purpose is to assess the effects of microwave radiation on the health of Naval personnel by taking advantage of marked occupational differences in level of exposure, and the availability of the extensive medical information recorded during service and subsequently in the veteran period.

Since World War II the Navy has maintained technical schools where tens of thousands of enlisted men have been trained in the use of radar for navigation and aiming weapons, and in the maintenance of the equipment. Other schools have graduated even larger numbers of rated technicians similarly selected for educational achievement, general intelligence, and motivation for technical work, thus offering the possibility of selecting valid comparison groups. The records of technical schools are available at the Military Personnel Records Center in St. Louis, Missouri, and they uniformly identify graduates of particular courses.

This study, then, is one that utilizes only extant records. It includes mortality, morbidity, both in service and later in Veterans Administration facilities, and requests for disability compensation, both granted and disallowed. The comparisons will be made for occupationally exposed men versus men not occupationally exposed who, for brevity, we shall call "controls." An exposure index has been devised for the occupationally exposed group (Table 1). We shall report on only the mortality data today, and we should state that these data together with the information on exposure morbidity and disability will be reported on in greater detail at a later time. A study such as this one, aimed at a wide variety of health effects, requires the follow-up of a sufficiently large number of men over a period of time long enough for effects to develop. The Korean War period was chosen and a study population of approximately 40,000 men, equally split between cases and controls, was sought (Table 2).

Table 1.

Records Study	
Mortality	1955-1974
Morbidity (In-Service)	1950-1959
Morbidity (VA)	1963-76
Disability	1976
Occupationally Exposed vs. Controls	
Radar Exposure Index	

Table 2. Exposure cohorts

Occupationally Exposed (Equipment Repair)	
Electronics Technician (ET)	12,967
Fire Control Technician (FT)	3,270
Aircraft Electronics Technician (AT)	3,728
	19,965
Controls (Equipment Operation)	
Radioman (RM)	9,221
Radarman (RD)	10,096
Aircraft Electrician's Mate (AE)	1,409
	20,726

The two exposure cohorts are each composed of three Naval Enlisted Classifications. The occupationally exposed cohort is made up of Electronics Technicians, Fire Control Technicians, and Aircraft Electronics Technicians, all of whom were trained in equipment repair. The controls, men trained in equipment operation, consist of Radiomen, Radarmen, and Aircraft Electricians Mates. The choice of occupations was a consensus decision on the part of Navy personnel involved in training and operations. The numbers of men finally eligible for the study are shown at the right. The men who did not serve in aircraft are all graduates of Class A schools during the period 1950 through 1954. The vast majority of the men serving in aircraft also graduated from Class A schools during this period, but these men also served at some time in an airborne patrol squadron.

The cohorts were composed predominantly of white males who entered service shortly after graduating from high school. The controls are, on the average, about one and one-half years younger (Table 3).

Table 3. Year of birth

Year	Occupationally Exposed (Percent)	Controls (Percent)
≤1915	2.1	0.6
1916-1920	2.6	0.6
1916-1925	5.4	2.3
1926-1930	32.5	22.5
1931-1935	56.8	72.7
≥1936	0.6	1.2
	100.0	100.0
Average YOB	1930.0	1931.4

Mortality was ascertained through the VA Beneficiary Identification and Records Locator Subsystem (BIRLS), a computerized index to veteran contact with that organization. The mortality of substantially all war veterans, regardless of any prior association with VA, is a matter of record in VA files. Certified cause of death was obtained from the application for death benefits, which usually includes a copy of the death certificate. We estimate that mortality information is retrievable for about 95 percent of Korean War veterans who have died (Table 4).

Table 4. Total mortality

	Occupationally Exposed		Controls			P
	OBS.	EXP.*	OBS.	EXP.*	Risk Ratio	(RR = 1)
All Trauma Disease	295	375.8	247	396.6	1.26	< 0.01
All Disease	311	561.5	321	519.1	0.90	NS
Unknown	13	-	11	-	-	-
Total	619	937.3	579	915.7	1.04	NS

*From age-specific U. S. white male death rates
NS:P > .05

Let me say a word about the format of the remaining tables. The control cohort was, you recall, on the average, about a year and a half younger than the occupationally exposed group. The risk ratios shown are the result of indirect age-standardization, in which the ratio of deaths observed and those expected on the basis of age-specific U. S. white male rates was calculated separately for each roster, and these were then divided to yield the risk ratio. Statistical tests were calculated according to the method of Haldane.(6)

Total mortality is summarized in this table. The first thing to note, I believe, is that, compared to the U. S. white male population of the same ages, these men have had a favorable mortality experience. This is to be expected because they were screened before entering service. Mortality is 66 percent of that expected for the occupationally exposed group and 63 percent of expected for the controls. There is a small but significant increase in trauma mortality in the occupationally exposed group as compared with the controls, but neither mortality from all diseases nor total mortality was significantly different in the two groups.

Taking a closer look at trauma mortality, I have broken it down by Accidents (motor vehicle and other), Suicide, and Homicide (Table 5). Three significant increases in risk of mortality are noted: Total, Accidents total, and Accidents other. Note that only in Other Accidents is the number of observed deaths greater than expected on the basis of U. S. rates, and this only in the occupationally exposed cohort. Examination of death certificates and other mortality information on men whose causes of death fell in this category showed that many of these men died in military aircraft accidents after the Korean War was over. We believe that the excessive number of deaths in aircraft accidents among the occupationally exposed men resulted from the fact that a larger proportion of them later became flying officers. More than twice as many became commissioned officers as did controls--5.3 versus 2.3 percent.

Table 5. Trauma mortality

	Occupationally Exposed		Controls			P
	OBS.	EXP.*	OBS.	EXP.*	Risk Ratio	(RR = 1)
Total	295	375.8	247	396.6	1.26	< 0.01
Accidents	225	271.6	174	289.9	1.38	< 0.01
Motor Vehicle	95	154.7	104	168.2	0.99	NS
Other	130	116.9	70	121.6	1.93	< 0.01
Suicide	49	68.5	51	69.2	0.97	NS
Homicide	13	31.2	14	32.8	0.98	NS

*From age-specific U. S. white male death rates
NS: P > .05

Deaths from motor vehicle accidents, suicide, and homicide are not elevated and are quite consistent between the two groups.

Turning now to disease mortality, we note first that in each category the numbers of deaths observed in the study cohorts are less than would have been expected at prevailing rates in the U. S. white male population (Table 6).

Table 6. Disease mortality

ICD 8th Rev. Code	Cause of Death	Occupationally Exposed OBS.	EXP.*	Controls OBS.	EXP.*	Risk Ratio	P (RR = 1)
000-796	All Disease	311	561.5	321	519.1	0.90	NS
140-209	All Malignant Neoplasms	96	127.4	84	119.1	1.07	NS
390-448	Cardiovascular	150	243.5	164	216.4	0.81	NS
430-438	Vascular Lesions of CNS	24	27.4	17	25.6	1.32	NS
410-413	Arteriosclerotic Heart Disease Other Cardiovascular	8	45.5	26	42.7	0.29	< 0.05
582-584	Chronic Nephritis, Other Renal	6	9.6	1	9.6	6.00	NS
470-486	Influenza and Pneumonia	9	17.3	4	16.8	1.26	NS
571	Cirrhosis of the Liver	18	37.3	15	34.1	1.10	NS

*From age-specific U. S. white male deaths rates
NS: P > .05

Mortality from all malignant neoplasms is increased in the occupationally exposed group (96 vs. 84) but not significantly so. Cardiovascular disease has a risk ratio of less than unity. Vascular lesions of the central nervous system, that is, strokes are elevated, but not significantly. The risk ratio for arteriosclerotic heart disease is less than unity. "Other" heart disease, primarily ICD 420-429 is significantly elevated in the control group. The causes of death are spread over the entire rubric, and we are seeking more information about these deaths. Mortality from chronic nephritis, as well as from influenza and pneumonia and cirrhosis is elevated in the occupationally exposed men, but not significantly so.

The numbers of deaths from specific malignant neoplasms are shown here (Table 7). Increased mortality in the occupationally exposed group is noted for neoplasms of the digestive organs (20 vs. 12), the respiratory tract (25 vs. 17), and leukemia (12 vs. 7), but none of the increases is statistically significant.

Table 7. Cancer mortality

ICD 8th Rev. Code	Cause of Death	Occupationally Exposed OBS.	Occupationally Exposed EXP.*	Controls OBS.	Controls EXP.*	Risk Ratio	P (RR = 1)
140-209	All Malignant Neoplasms	96	127.4	84	119.1	1.07	NS
140-149	Buccal Cavity and Pharynx	2	2.8	2	2.5	0.89	NS
150-159	Digestive Organs	20	24.1	12	21.6	1.49	NS
160-163	Respiratory Tract	25	31.0	17	26.9	1.28	NS
180-187	Genital Organs	5	6.6	5	7.7	1.06	NS
188-189	Urinary Organs	2	2.4	4	1.4	0.29	NS
204-207	Leukemia and Aleukemia	12	11.2	7	11.3	1.72	NS
200-203, 208-209	Other Lymphatic	12	18.2	12	18.3	1.00	NS
	Other	18	29.2	25	28.2	0.70	NS

*From age-specific U. S. white male death rates
NS: P > .05

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OPEN DISCUSSION

DR. OSEPCHUK: How do you determine that the two classes of occupations--the low exposure group as compared to the high exposure group--are high or low exposure?

DR. ROBINETTE: We have no dosimetry measurements; this is an occupational study and these enlisted classifications were arrived at after discussions with Navy personnel involved in operations and training.

DR. OSEPCHUK: To make it clearer, maybe I missed something; but you are comparing the two groups, there is not a third control group?

DR. ROBINETTE: That is correct.

MR. WAXLER: I was an electronic technician in the Coast Guard some years ago. One of the hazards that was clearly present (as the case of being a radioman) was that you had to stick your hands inside a transmitter occasionally, and you could get an electric shock which would also add up to heart failure and lots of other things like that. So one possibility is simply that they were exposed to more wall sockets and things like that, as well as to radiation from the apparatus. Do you have any plans to pursue that kind of possibility?

DR. ROBINETTE: I feel that such possibilities apply equally to both groups.

MR. NEILL: For the index of exposure, whether it is high or low, was this based on the type of equipment, and was the length of potential exposure considered--whether a fellow worked there for 1 year or 3 years?

DR. ROBINETTE: Yes. This was based on the type of work the men were trained to do while they were in the Navy and on the equipment they used. Those data have not been analyzed yet, nor have the data in the exposure index that we have devised.

DR. TELLES: Can you tell us what sorts of criteria the Navy uses in determining whether an individual should be placed in a high or low exposure potential category? Was this based on their knowledge derived from actually having measured the potential for exposure in these individuals, or is this just some other basis?

DR. ROBINETTE: The Navy did not say anything about exposure high, low, or intermediate. These are categories which we have constructed, based on occupation. We did discuss this with the Navy, and the consensus was that men who repair the gear have more of a chance of being exposed than the men who operate the gear.

DR. TELLES: What is that based on?

DR. ROBINETTE: That is based on discussions with Navy specialists.

MR. RUGGERA: You defined high exposure and low exposure. Did you define this by frequency in any way?

DR. ROBINETTE: No, we defined it on the basis of the work the men were trained to do by the Navy. The technicians were assumed to have a better chance of being exposed than were the men who operated the equipment.

DR. LUNDIN: I would like to ask how powerful the study is at detecting the observed size difference in cancer mortality. For example, you are showing us 7 percent estimated excess which is nonstatistically significant, but how do we interpret that? Could there be an excess of 50 percent? Would one be expected to detect these size differences?

DR. ROBINETTE: We were after power in the neighborhood of 90 percent for a doubling of the cancer risk. The chance of detection of smaller differences in risk is less likely.

MR. DODGE: Will the study make any specific recommendations as to how follow-up studies of this sort might be conducted. What I have in mind is, will an effort be made to categorize more carefully on the basis of dose experience and duration of exposure of naval personnel or other military personnel?

DR. ROBINETTE: We certainly intend to do that for our study here as well as we can with this crude index to exposure which we have. These data are not ready at this time; all we have are the numbers.

MR. MUMFORD: Do you have data on the difference in the expectancy of life of ordinary civilians and the servicemen that you quoted?

DR. ROBINETTE: Only that the servicemen, in general, have a lower death rate, because they were medically screened before entering service. These were the population expectations that I was showing here for white males.

MR. MUMFORD: This is part of the general population that you showed, I presume?

DR. ROBINETTE: Yes, for the U.S. white male population of the same ages.

MR. MUMFORD: Yes, and you are saying that the servicemen, in general, exceed this?

DR. ROBINETTE: Yes, the servicemen have a longer life expectancy, a greater life expectancy.

FROM THE FLOOR: I did not get a chance to look at the magnitude of all your differences. Did you find anything aside from the relative risk where attributable risk may have suggested anything, even with the large N that you have where the attributable risk may make it worthwhile to look even further?

DR. ROBINETTE: Not so far, no.

EFFECTS OF REPEATED MICROWAVE IRRADIATIONS TO THE ALBINO RABBIT EYE[1]

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INTRODUCTION

Since the late 1940's and early 1950's much concern for the ocular effects of microwave irradiation has been present. With the introduction of multiple frequency, high energy microwave generating equipment, continued interest and controversy has precipitated renewed investigations into the potential problems of accidental injury. The cataractogenic properties of microwave irradiations is controversial. Our previous report (1) on the effects of single exposures to irradiations of increasing power density given to albino rabbits with follow-up for periods of one year, during which development of cataracts was not observed served as the basis for the present experiment.

MATERIALS AND METHODS

Adult New Zealand albino rabbits of approximately similar size and weight were used for this experiment. All eyes were examined with the ophthalmoscope and slit lamp, fully dilated, prior to irradiation to detect any pre existing corneal or lenticular abnormalities. All materials and methods, equipment and exposure techniques were identical with those described in our previously published report (1). The frequency of irradiation was 3000 MHz cw.

Exposures were given to the left eye of each animal for a 15 minute period each consecutive day for a total of 30 days (for those which survived the 30 day period). The power densities of irradiation used were 50, 100, 200, 300, 400, 500 mW/cm^2.

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[1] Supported in part by Research Grant No. FD00686 from the Food and Drug Administration, Dept. Health Education and Welfare. (Dr. Fine).

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army of the Department of Defense.

Clinical examination by slit lamp was carried out daily for the first thirty days and intermittently thereafter for up to one year. Selected animals were sacrificed for histologic study by both light and electron microscopy.

RESULTS

Table 1. Exposures

Power Density (15 exposure)	Original Animals	Animals surviving 30 exposures	Average exposure received by animal that died
50 mW/cm^2	5	5	---
100 "	5	4	22
200 "	5	4	23
300 "	5	4	18
400 "	5	1	8
500 "	10	3	4

As the power density increased, fewer of the animals survived the 30 day irradiation. The reasons for the deaths were not determined.

Table 2. Responses

Power Density (15 exposure)	Acute Changes	Chronic Changes
50 mW/cm^2	none	none
100 "	"	"
200 "	"	"
300 "	all developed iritis	PSI average 17-1/2 days
400 "		PSI average 10 days opacities avg. 15 days
500 "		PSI average 5 days vacuoles average 8 days opacities average 10 days

Below 300 mW/cm^2 no ocular changes were detected. At 300 mW/cm^2 and above, all eyes reacted ith congestion of the limbal vessels, pupillary constriction and evidence of iritis. Posterior subcapsular iridescence (PSI) developed an average of 17-1/2 days after irradiation at the 300 mW/cm^2 level. At 400 mW/cm^2 the PSI appeared at an average 5 days, vacuoles appeared in the posterior cortex of the lens at an average of 8 days and posterior cortical cataractuous changes at an average of 10 days.

The lenticular opacities progressed but were limited to the posterior cortex during the following year of observation. No other ocular changes were noted by our methods of examination.

DISCUSSION

It is clear from these results that production of lenticular changes is dependent on the power density of the exposure. No changes were produced below 200 mW/cm^2 for 15 minutes/day for 30 days. Posterior subcapsular changes and subsequent posterior cortical opacities appeared with all power levels above 300 mW/cm^2 x 15 min/day and much earlier at higher power levels. It is of interest that histologic examination (light and electromicroscopic) of posterior subcapsular iridescence revealed a vacuolar separation from the posterior capsule of the most posterior cortical lens cells. Similar changes were noted near the posterior pole of the lens. Temperature measurements by Kramar et al (2) have shown that maximum temperature elevations occur in the retrolental area adjacent to the posterior surface of the lens, suggesting that the changes occurring in the adjacent lens cortex are the result of heat generated in the immediate vicinity. Similar observations have been made for the anterior lens surface using intense incoherent white or infrared light.(3)

Anterior subcapsular cortical lens opacities can be produced where intense white light is absorbed by the pigment epithelium of the iris and there converted to heat. Similar anterior lens opacities are extremely difficult to produce in the albino where the iris neuroepithelial layer is not pigmented, or in the pigmented rabbit in the regions where the iris pigment epithelium does not make contact with the lens.

It appears, therefore, that the cataractuous changes are dependent on the production of local elevations of temperature sufficient to injure the lens cortical cells, which, due to their relative inability to dissipate the heat undergo first, reactive changes (intercellular vacuolation) and subsequently necrotic changes (cataractuous).

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OPEN DISCUSSION

DR. HIRSCH: Actually, this phenomena can be demonstrated with almost any slit lamp; the important part was not so much the light source as it was being able to have good axial illumination. If you want to talk to me later I'll show you how we can demonstrate it. Most opthalmologists are aware of it; it has been described in Berliner back in the 1940's. There is nothing new about it, but nobody really has had a good explanation for it.

MR. SIMON: It is seen commonly in almost all forms of senile cataract, and what I was alluding to is whether or not you have used a halogen light source.

DR. HIRSCH: No, it was a standard size one but the significance is questionable. We do feel that at least in the one year period of observation where the animals had no posterior subcapsule iridescence prior to exposure that it was one of the first things that we saw in the irradiated eye; and it did act, at least we felt, as a prescursor to the vacuole formation and the actual lens' opacities. But clinically, opthalmologists have known for years that when they see a lot of this posterior subcapsule iridescence or play of colors, that often they have the impression that they are going to have posterior subcapsule cataracts in the future. Now the time period is quite variable; we see it quite often in younger people.

We did a clinical survey which I really did not come prepared to talk about, in which there was no question that posterior subcapsule iridescence was present in younger aged people; but it increased with age along with the vacuole formation and the opacifications.

MR. SIMON: Can you tell me what your earliest ultrastructural or fine constructural changes were in those mialin figures preceding the development of these vacuoles or any change in the density of the matrix in the fibrocells themselves?

DR. HIRSCH: I really am not prepared to talk about that, the earliest ones that we sacrificed had clinical vacuole formation; so we did not really look at lenses before they had morphologic changes.

DR. KANTOR: What was your distance of the rabbit's eye from the focus.

DR. HIRSCH: It was one foot beyond the focal point. Pete Brown is in the audience if you want to direct the technical questions to him.

DR. KANTOR: It is a general question. Did you have a chance to vary the distance to see that obviously the closer you get to the focus, the more you get the near field situation? Did you have an opportunity to study the spacial dependence of cataract formation in the terms of distance from the focus to the eye exposed?

DR. HIRSCH: We did not vary it, but Pete will answer the question.

MR. BROWN: The reason we went one foot behind the focus was because even though we used Ketamine to knock the animals out, they still moved around a little bit; so if we had them right at the focus, they could easily just fall or slip out. The focus focussed to such a small field that we had to back up a foot so they would not slip. We did not want to restrain them with any Plexiglas because that would distort the field. We had them sitting on styrofoam in an anachoid chamber, essentially.

DR. KANTOR: I do not recall Dr. Carpenter's spacing when he used his applicator; if it included spacing, his regimen would be different than yours.

MR. BROWN: Yes, it probably would be.

DR. KANTOR: If you were to compare the two exposure results, I would have to find out if the field distributions would be quite different.

MR. BROWN: I am sure they would be.

DR. CARPENTER: I think that the point that Mr. Brown just brought up is important. After all, when you are measuring power density in a complex field, particularly the near field of this director or of a focus field, your instrument is sort of rubbery. It contracts or expands, and you do not get the same measurement in one place as another. You said 300 milliwatts is a threshhold; but we have produced a lot of cataracts at 180 milliwatts. However, if you measure it with a NARDA 8100 at a 2 inch distance, you will not get the same figure of power density that you do with a 8323 probe with the broad band NARDA. There is a radial component that the 8100 does not measure. I always take, with at least 3 grains of salt, most measurements of power density including my own, and then we try to repeat them and at least get in the same part of the ball park.

DR. HIRSCH: I think we would agree, though, that if we do not want to talk in terms of specific numbers, these are high levels of microwave or radiation. I think that is the important part.

DR. CARPENTER: We did get at one time (and it has been recorded) accumulative consecutive 20 radiations, one a day, at a level which we measured at 180 milliwatts at the age of 23; but at least the animal did not need to be anesthetized and seemed to enjoy the experience.

DR. ALBERT: You talked about the lengths which are alright; but with these power levels, what is happening to the neural elements of the retina? I think they are cooked by this time. The cataract may not have appeared, but the visual system and the nervous system is probably done for.

DR. HIRSCH: I have no idea. I can tell you morphologically, at least with an ophthalmoscope that the architecture looked normal; we did not see, for instance, hemorrhages. However, we did not look specifically histologically at those areas.

DR. ALBERT: Do you know if Dr. Carpenter is going to speak about that?

DR. HIRSCH: I do not know.

DR. MICHAELSON: An interesting point came up in this discussion between Dr. Carpenter and Dr. Hirsch, and I am not sure if you spoke of the difference between the anesthetized rabbit and the nonanesthetized rabbit; but I think this is a very critical point. As I understand it, you mentioned that in the unanesthetized rabbit you did not get a cataract.

DR. HIRSCH: No, what we did was some preliminary work on nonanesthetized animals; and we showed that at these power levels, we could not do the experiment because they were intolerant to the heat produced and just walked out of the field. I think this is significant if you are talking in terms of safety standards. What we are showing is that the heat generated is way above the cutaneous sensation level and that in order to produce the cataract, you have to anesthetize the animals in order to keep them there long enough to do it.

DR. MICHAELSON: Yes, I think all of us have seen the same thing. I believe in monkeys now, at Tulane, they are finding the same thing--that the pain or the irritations are enough to prevent exposure to the animal, thus preventing the formation of a cataract.

DR. HIRSCH: Yes, but it was pretty high.

DR. ALBERT: Just in case I did not make myself clear, what I wanted to know was, could the rabbit see even though he did not have a cataract? Maybe we could leave the histology aside.

DR. HIRSCH: That is very difficult to answer; my "carrot chart" was out that day, so I really cannot be sure, but it was interesting in the other talk. I did not notice any nystagmus, and I facetiously said this to Pete during it. But during all these exposures over the years, I never noticed nystagmus in rabbits. I do not know how to evaluate whether they can see; they seem to be aware that we are going to, for instance, examine them--the usual autonomic responses that any investigators in this field are aware of were present when we walked into the room. However, I think that if the retina were fine, there would be no reason that these type of lens opacities would be totally blocking light. We know from clinical experiences that even with the densest cataracts there is still light perception.

DR. ROSENTHAL: We did some experiments wherein we took an eye, for example, and put it up against the waveguide and pointed energy into it and tried to pick up the energy in back of the eye, and we could not. We got the impression that it never actually reached the retina, rather that it all was absorbed; it was a very good absorber.

HISTOPATHOLOGICAL CHANGES DURING DEVELOPMENT OF MICROWAVE CATARACTS

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ABSTRACT

Histopathological changes associated with the development of microwave cataracts were studied in serial sections of rabbit eye lenses preserved at post-irradiation intervals ranging from 12 hours to 123 days. Findings may be summarized as follows:

During the first 2 days post-irradiation, lens fibers in the posterior subcapsular cortex become hydropic, with slight swelling or with many tiny vesicles. This is interpreted as an initial response to microwave heating. During the fourth day, epithelial cells have migrated from the equator posteriorly under the capsule and numerous mitotic figures indicate proliferative activity. In the ensuing 5 days, swollen "balloon cells" appear at the equator or in the posterior cortex; these appear to represent abortive attempts of the cells to differentiate into new lens fibers as they normally do. The posterior migration of epithelial cells and transformation of some of them into "balloon cells" have been described previously as a characteristic response to ionizing radiation. By 3 to 5 weeks post-irradiation, there are many nucleated cells and fibers in the posterior cortex, as well as cysts containing debris of protein precipitates and broken down cells. Regions of highly swollen or denatured fibers appear to be the histopathological representation of opacities. These conditions remain unchanged at 123 days post-irradiation. The histopathological changes noted here are not representative of lens changes induced by heating.

EDITOR'S NOTE

At the author's request, we are providing an abstract only for this presentation. The presentation was a description of a long series of slides. Dr. Carpenter felt that many of the described changes were of such a subtle nature that they would be lost in reproduction.

ARE MICROWAVE CATARACTS THERMALLY CAUSED?*

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ABSTRACT

If sufficient in power and duration, 2.45 GHz radiation causes in the rabbit eye (a) an immediate increase in intraocular temperature and (b) after a latent period of a few days, development of opacities in the posterior subcapsular cortex of the lens. To test whether the first event is the cause of the second, two experimental approaches were utilized. In one, external application of heat to the eye induced the same increase in intraocular temperature over the same duration of time as does a cataractogenic microwave exposure. No posterior cataracts developed in 5 of 6 such experiments. In other experiments, loss of body heat was restricted by encasing the animal's ears in an insulated plastic sack through which flowed air heated to 55° C. By simultaneously irradiating one eye, it became possible to elevate both retrolental and rectal temperatures to levels characteristic of a cataractogenic microwave irradiation, even though microwave power applied to the eye during the 40-minute exposure was as much as 25 percent below the 250 mW/cm^2 level necessary for cataract induction. If cataracts were solely of thermal origin, they should have been induced in all animals so treated. Actually, posterior cortical cataracts occurred in only 3 of 10 experiments. We conclude that the increase in intraocular temperature occurring during microwave irradiation is not the sole causative factor in microwave cataractogenesis.

INTRODUCTION

When the eye of a rabbit is exposed to 2.45 GHz radiation of sufficient power density and for an appropriate period of time, there are two demonstrable effects. The first occurs immediately and consists of an increase in intraocular temper ature, the amount being largely dependent upon the power density of the incident radiation; upon cessation of irradiation, the intraocular temperature gradually returns to normal. The second effect is a delayed response, the development of opacities in the lens. From 2 to 4 days may elapse before the opacities begin to become apparent and this effect is an irreversible one.

Does the first response evoke the second? Are microwave-induced lens opacities (cataracts) thermally caused? The sequential juxtaposition of the two responses has frequently led to the assumption that they represent a cause and effect relationship (**1-8**) and that the microwave-induced heating of the eye is solely the cause of the lens opacities that subsequently develop. This view has been expressed briefly in the statement: "Certainly we know that microwaves can produce thermal damage such as cataracts."(9)

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*Partial support furnished by Naval Medical Research and Development Command.

Because the lens seems to be particularly susceptible to damage by microwaves, some authors have offered the explanation that the vascular system of the eye is inadequate for carrying heat from the organ. The following statements, quoted from the literature, are representative:

"Due to a relatively poor blood supply, the eye's ability to conduct heat away from it is very poor. In particular, the lens has no cooling system..." (**10**)

"The eye, with its poor blood supply, particularly around the lens, is susceptible to cataract formation in the microwave range that can penetrate to that depth (wavelength of 10 to 12.5 cm.)" (**11**)

"The eye and the testicles are most susceptible to microwave heating since these organs are ischemic and must rely on other means of thermoregulation." (**12**) (It may be appropriate to mention here that far from being ischemic, the testes have the rich blood capillary supply that is a structural characteristic of all endocrine glands.)

These statements suggest a lack of knowledge as to the structure of the eye. Mammalian eyes typically are generously supplied with blood. This is definitely the case with respect to the rabbit and the dog, whose eyes have been the subject of microwave experiments. It is also true of the human eye, which is of particular concern in any consideration of microwave radiation as a health hazard. Indeed, the marked diminution in the caliber of the human internal carotid artery immediately after it has given off the ophthalmic artery is an anatomical reminder of the considerable volume of blood constantly being supplied to the eye and its associated structures.

In some cases, authors have emphasized that the avascularity of the lens renders it unable to dissipate heat. For example:

"The lens of the eye lacks an efficient circulatory system, thus it cannot dissipate heat as readily as the rest of the body and, for this reason, may be injured more easily than the remainder of the organism." (**13**)

"The lens is avascular and is located at least 2 mm or more from any blood supply, making it much less effective in dissipating heat as compared with most other tissues and organs." (**1**)

The lens is an epithelial derivative and, in common with all epithelia, has no intrinsic blood supply and must depend on nearby capillaries for its metabolic needs. It is suspended in place by its suspensory ligament attachments to the adjacent ciliary body. The anterior portion of the ciliary body, which encircles the equator of the lens, bears a series of parallel folds, the ciliary processes. There are some 70 of these in the human eye and approximately 100 in the rabbit, in which species they may extend over the posterior face of the iris to within 1 mm of the pupil (**14**). In each ciliary process, there is a rich network of capillaries supplied by a small artery from in front and drained by small veins leading back into the choroid. The circumlental space between the lens equator and these highly vascularized processes is only 0.5 mm wide in the human eye and is little more than that in the rabbit and the dog. The vascular iris spans the front of the lens, separated from it by the narrow (1 mm or less) space of the posterior chamber. The pupillary region of the iris commonly is in contact with the lens.

It is apparent, therefore, that the lens is not "located at least 2 mm or more from any blood supply." Rather, both its anterior surface and equator are quite close to the rich vascular beds of the iris and the ciliary processes.

Furthermore, in the slender space of the posterior chamber which intervenes, there is a constant flow of aqueous humor passing from the ciliary processes through the pupil into the anterior chamber, from which it is drained at a rate of approximately 2 to 4 μl/min. (**15**). (Dr. W. Morton Grant, personal communication.)

The posterior lens surface rests against the anterior face of the gel-like avascular vitreous body. Schwartz and Feller (**16**) in a study of the albino rabbit eye, found the greatest temperature gradient within the eye to be from the anterior lens surface to the midvitreous humor, with little change in temperature gradient in the vitreous body, "probably because the vitreous is surrounded by a shell of vascular tissue that radiates heat and maintains a homogeneous temperature." The maximal temperature response to microwave irradiation of the eye occurs in the vitreous just behind the posterior pole of the lens (**17,18**).

The frequently voiced claim that the eye cannot effectively dissipate heat can be demonstrated to be untenable by a simple experiment. If the temperature at the posterior pole of the lens in an anesthetized rabbit is measured prior to and during microwave irradiation, it may be found to rise perhaps 5° C in the course of a 15-minute exposure. If a lethal dose of anesthetic is then injected intravenously, the heart will stop beating, whereupon the intraocular temperature will rapidly rise another 10° C, thus indicating that the vascular system is capable of handling at least two-thirds of the thermal stress which radiation imposes upon the eye.

Evidence supporting the contention that microwave cataracts are purely a thermal effect is scant but there are two relevant published reports. Baillie (**7**) designed experiments "to determine whether or not a cumulative microwave cataract could be produced when the exposures were made under sufficient hypothermia to prevent the lens temperature from exceeding normal body temperature." He reasoned that development of a cataract under these conditions would support the hypothesis of a non-thermal cataractogenic effect. Conversely, failure to develop would argue for a thermal origin.

Exposures were carried out on the eyes of dogs, with the animal in a large aluminum box placed inside what was essentially a large microwave oven operating at 2.5 GHz. A small hole in the upper surface of the box allowed leakage of some of the energized cavity's field into it, where the anesthetized dog was placed with one eye positioned under the hole and held there by sutures. The power density measured at that point was 5 watts/cm^2. In five experiments to establish a cataractogenic threshold, Baillie found this power density was sufficient to produce prompt thermal coagulation of the lens substance during a single exposure lasting 75 seconds but had no apparent effect after a 55-second exposure. The cataractogenic threshold was taken to be 60 seconds.

In two dogs, Baillie irradiated the right eyes for 30 and 35 seconds respectively, after which their bodies were cooled to 22° C by immersing them in ice water and the left eyes were given the same exposure. This procedure was repeated every few days for a total of seven exposures of each eye in 15 days, after which the dogs were kept for an observation period of 14 days, with daily examination of the eyes. In both animals, dense cataracts developed in the normothermic right eyes while the left eyes irradiated during hypothermia remained clear. In two more experiments, the right eye received ten consecutive exposures, each of 40- or 45-seconds duration, during a little more than 1-3/4 hours, with a 10-minute period between exposures to permit the intraocular temperature to return to normal. The same procedure was employed in irradiating the left eye but the body temperature was lowered to 22° C by immersing it in ice water. As in the other two experiments, both dogs developed cataracts in the normothermic right eyes but not in the left eyes irradiated under hypothermic conditions. On the basis of these experiments, Baillie concluded that microwave cataractogenesis is, directly or indirectly, a thermal phenomenom occurring when the intraocular temperature exceeds 43° C.

Experiments of somewhat similar nature were reported by Kramar et al. (**19,20**). They had previously determined (**21**) cataractogenic thresholds when rabbits were irradiated in the near zone of a 2450 MHz corner reflector (diathermy "C" director) with the corneal surface of the eye placed 5 cm from the crossing point of the dipole feed. Under Nembutal anesthesia, the animals were positioned in an acrylic box, with head and extremities outside and the head fixated by an acrylic restrainer. Incident power density was measured with a Narda 8100 electromagnetic radiation monitor at the position of the eye with the box in place but the animal absent. They found a minimal cataractogenic dose to be 150 mW/cm^2 for 100 minutes.

These investigators also examined intraocular temperatures (**20**) induced by the radiation and concluded that a retrolental temperature above 41° C was necessary for the development of lens opacities and that a critical temperature for cataractogenesis was approximately 43° C. they then considered the hypothesis that "If upon exposure to a source of radiation which produces local temperature elevation in the eye, if any portion of the lens achieves sufficiently high temperature, a cataract will form" and designed experiments to test the reciprocal of it, which might be stated as: A level of radiation normally adequate to cause formation of a cataract will be ineffective if the temperature of the lens is prevented from reaching a sufficiently high temperature. They selected 41° C as the allowable maximal intraocular temperature, measured retrolentally in the vitreous body. In a series of preliminary experiments, they subjected anesthetized rabbits to cataractogenic exposures while maintaining them in a hypothermic condition in ice water. Thermocouple measurements showed retrolental temperatures well below 41° C (36.6° to 39°), with accompanying rectal temperatures of 25° C to 31° C.

Nine experiments of this nature were then performed with only the rectal temperature monitored and with exposures at incident power densities of 200 mW/cm^2 for 30 minutes, 300 mW/cm^2 for 20 minutes, and 400 mW/cm^2 for 15 minutes. In no case did lens opacities develop during a 2- to 3-months' observation period. The conclusion was drawn that heat alone is responsible for damage to the lens following single, high-level irradiation.

Carrying the experiments a step further, Kramar et al. (**22**) subjected rabbits to conditions of general hyperthemia sufficient to raise the retrolental temperature to a level known to be associated with cataract formation, which they considered to be 42° C or above for at least 30 minutes. If cataracts developed under these conditions, then heat alone could be held responsible for the lens damage. Surprisingly, no detectable lens abnormalities were found during ensuing periodic examinations of the eye. The question of whether microwave cataracts were thermal or non-thermal in origin remained equivocal and vexing.

We also addressed the question, but from two different approaches. In one (**23**), instead of producing general hyperthermia to raise the retrolental temperature, we selectively heated the anterior segment of the eye by direct application of heat to that part of the sclera overlying the ciliary body, thus heating the blood flowing in the iris in front of the lens and in the ciliary body encircling its equator. Our second approach took advantage of the fact that convection from the rabbit's large ears is its chief means of heat loss. By raising the ear temperature while subjecting one eye to microwave exposure of subthreshold power density, we attempted to provide a cataractogenic retrolental temperature coincident with subthreshold irradiation.

MATERIALS AND METHODS

For selectively applying external heat to the eye, we devised a thin, concave, copper collar shaped to conform to the contour of the scleral region of the anterior segment. Soldered to its upper convex surface was a thin circular brass tube through which hot water was circulated from a large thermostatically regulated water bath (Fig. 1). The upper surfaces of the copper collar and the brass tubing, as well as all plastic tubing conducting hot water to it, were covered with open cell spongy urethane for insulation against heat loss. The rabbit, anesthetized by sodium pentobarbitol administered intravenously, lay on its side with the heating device carefully positioned on the proptosed eye by means of a mechanical manipulator (Fig. 2). Water dripping on the cornea at a rate of 3 drops per minute prevented drying. In a series of preliminary experiments, retrolental temperatures were monitored by means of a 0.009-inch diameter Bailey IT-2 themocouple probe inserted in the eye so that its junction was in the vitreous body just behind the posterior pole of the lens, where its position could be verified by ophthalmoscopy. In some instances, aqueous humor temperature was taken by a similar probe placed in the anterior chamber of the eye. Rectal temperature was monitored by a Bailey RET-1 probe inserted rectally a distance of 5 inches. Temperatures from all three probes were displayed on a Bailey BAT-8 digital thermometer.

In a second set of experiments, heat loss by convection from the ears was prevented by enclosing them in a 7- by 4-inch polyethylene sack, with hot air entering one end of it from a 1/4-inch tygon tubing (Fig. 3). The air was heated as it passed through a 5-inch diameter 10 turn coil of 1/4-inch copper tubing immersed in a regulated water bath. Heat insulation for the sack and tygon tubing supplying it was provided by a thickness of open cell spongy urethane (Fig. 4).

The radiation source was a Raytheon Model CMD-4 Microtherm diathermy unit operating at 2450 MHz and rated at 125 watts at full power. Its output was led through an isolator and two directional couplers to a Director "C" dipole antenna with corner reflector, which was in an anechoic chamber. The eye was positioned 5 cm from the crossing point of the dipole feed. Reflected power was tuned out by a double stub tuner in the line. Forward going power was monitored by a Keithley Model 168 autoranging digital multimeter plugged into the Hewlett Packard Model 431-B microwave power meter connected to one of the directional couplers.

For the purpose of ascertaining whether a thermocouple in the eye was affected by the electric field, two special devices were constructed. One, which we termed an Adjustable Propelled Thermocouple (APT), was made in part from an automatic blood lancet having a bell tip (Clay-Adams No. A-2562/X) (Fig. 5). This was modified by substituting for the spring powered lancet a narrow tube consisting of three segments: a 15-mm length with the sharp end of an 18 ga. hypodermic needle and an 83-mm length of a Wintrobe cannula (Clay-Adams No. A2453), the two joined together by a 7-mm section of nylon machined to a diameter of 2.75 mm which served as a thermal insulator to prevent heat conduction from the hypodermic needle. In the open end of the needle was a Teflon-sheathed thermocouple 0.025 inches in diameter; its lead passed through the tubular unit to a Bailey BAT-8 digital thermometer. In use, the center tube bearing the thermocouple was retracted, thus compressing the spring. The bell tip, adjusted for the desired depth of penetration, was held against the eyeball, the trigger

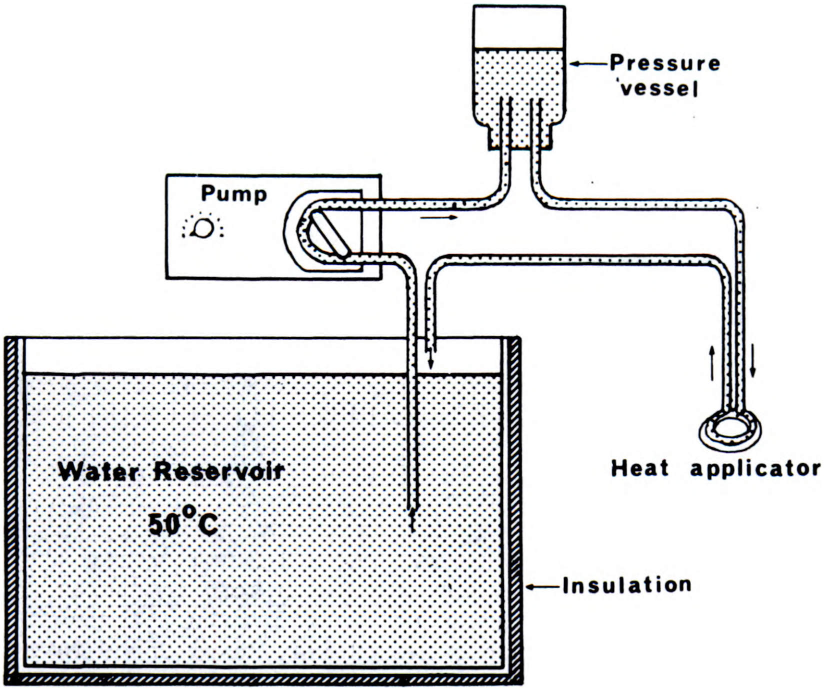
Pressure vessel
Pump
Water Reservoir
50°C
Heat applicator
Insulation

Figure 1. Diagrammatic representation of device for external application of heat to the eye.

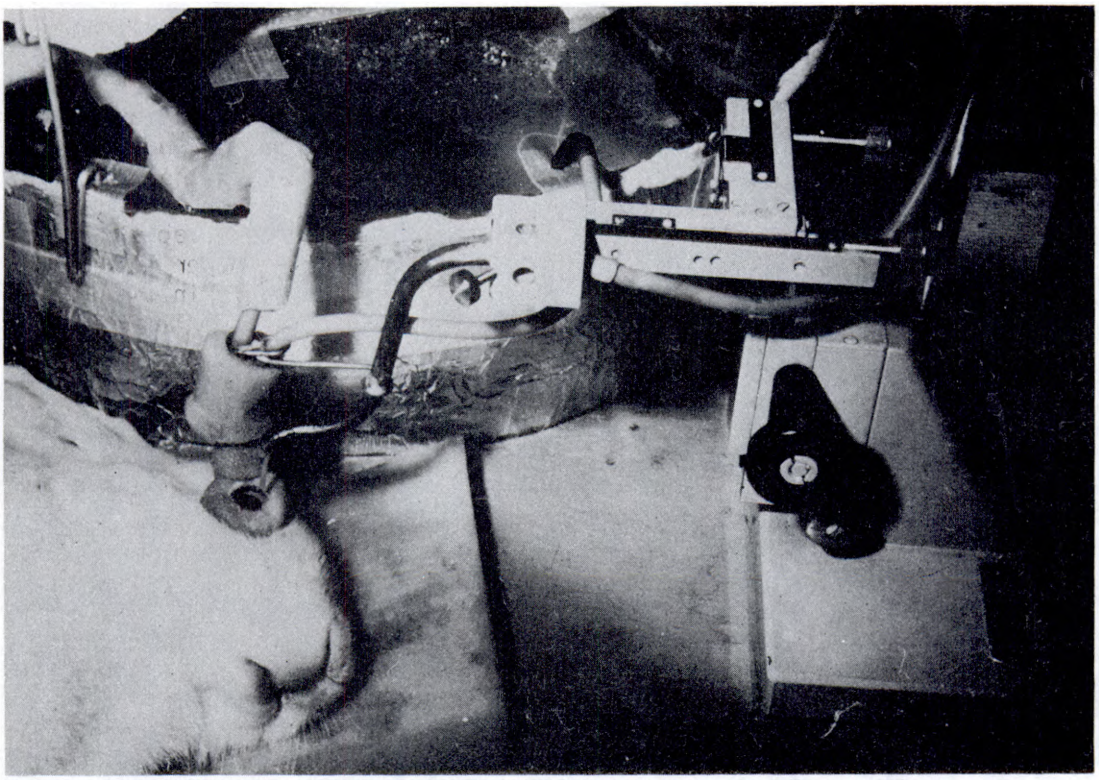

Figure 2. Rabbit with external heat applicator in position on left eye.

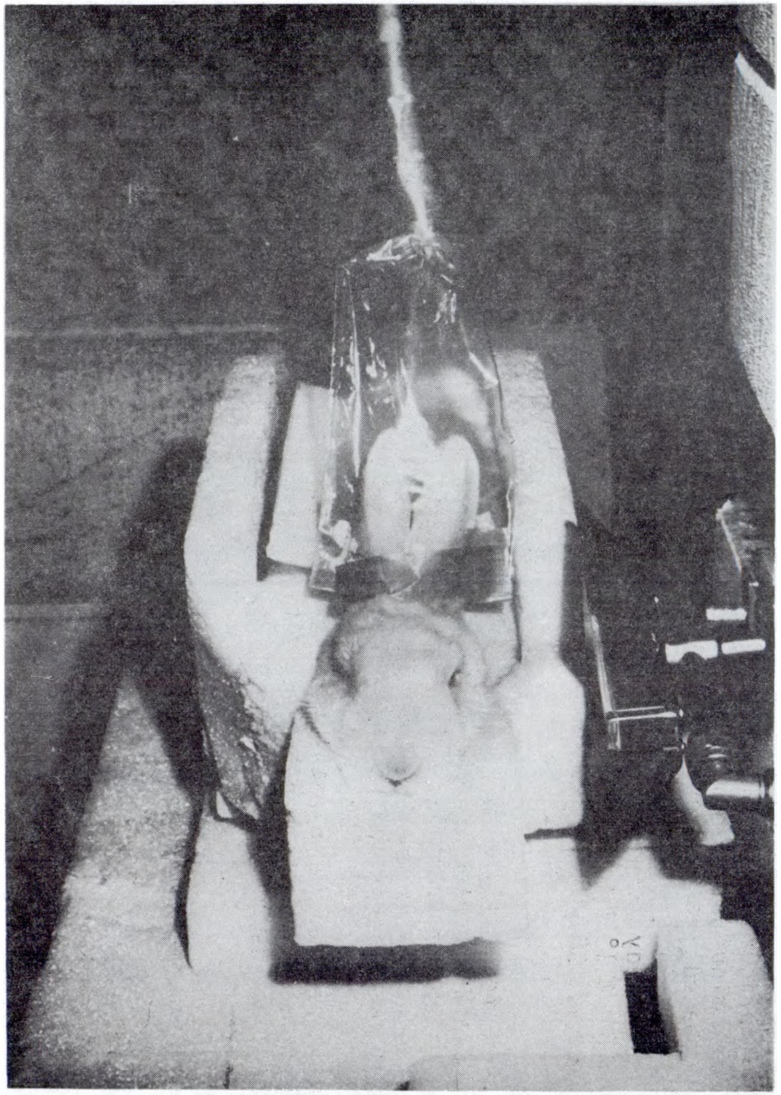

Figure 3. Rabbit with ears enclosed in plastic air sack.

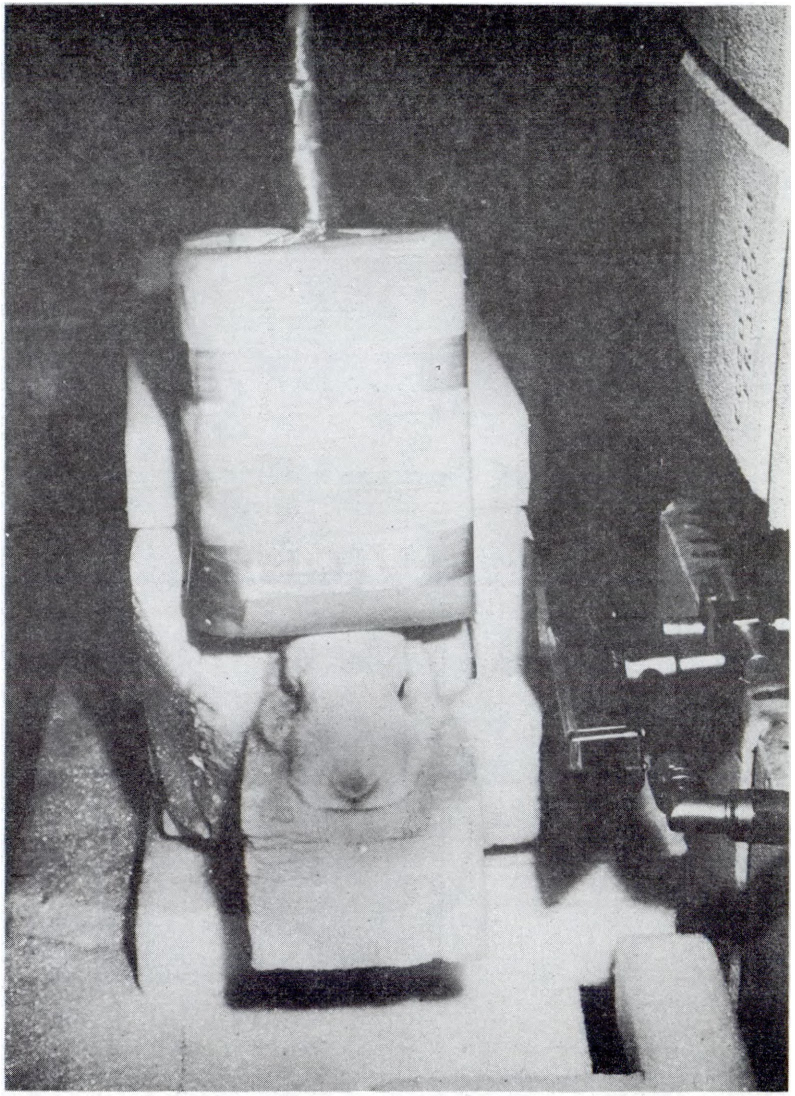

Figure 4. Rabbit with ears enclosed in plastic air sack, insulation in place.

was pressed, and the thermocouple was instantly propelled to the desired position in the eye.

The second piece of equipment was designed to test whether two identical thermocouples under the same temperature conditions would give the same readings when one was in a microwave field and the other was not. It consisted of a vertical 18-inch disc of polystyrene foam (Styrofoam), 4-inches thick, bearing a groove in its vertical diameter to accommodate a length of 1/4-inch tygon tubing (Fig. 6). Tygon sheathed thermocouples were inserted into the tubing, one (A) situated 4 inches from the lower end of the groove and the other (B) at its upper end. The thermocouple leads passed through the tubing wall, that of Thermocouple A running upward along the tubing. A second 2-inch thick Styrofoam disc formed an insulating cover and the discs together fitted into a circular opening in a 6-inch thick Styrofoam square. When placed in position in front of the Director "C," Thermocouple A was directly in front of and 2-inches distant from the crossing point of the dipole feed and Thermocouple B was outside the field. Warm water was circulated through the tubing from a constant temperature bath and a Bailey BAT-8 digitial thermometer displayed the temperature readings.

RESULTS

Raising the Retrolental Temperature by Direct Application of Heat to the Eye

Before attempting to raise the retrolental temperature to a level characteristic of a cataractogenic microwave exposure, we first had to identify that exposure in terms of incident power density and duration. This was done by testing in 19 different animals the effect of exposing the eye to an incident power density of 250 mW/cm^2 continuous wave for 20, 30, 35, and 40 minutes, respectively (Table 1). The 40-minute irradiation caused posterior subcapsular cataracts to form in 5 of 6 cases and was adopted as a cataractogenic dose.

We next measured retrolental temperature changes associated with this dose. Thermocouple measurements of retrolental temperatures during irradiation are shown in figure 7 for three rabbits. The temperature rose rapidly during the first 10 minutes, as much as 5.7° to 7.4° C, after which it became stabilized and increased approximately only another half degree during the remaining 30 minutes. In the two animals in which the initial retrolental temperature was 37°, the steady temperature during the final half four was 44 ± 0.2° in one case and 44.8 ± 0.1° in the other. In the third animal, the initial retrolental temperature was 35.7°. It reached 41.4° in 10 minutes and during the next half hour settled at 42.1 ± 0.2°.

The feasibility of attempting to replicate the temperature by direct application of heat to the external surface of the eye was tested by applying the heated copper collar to the eyes of anesthetized rabbits after insertion of a thermocouple in the retrolental position. Measurements were recorded every minute for 30 minutes at three different water bath temperatures: 53°, 51.5°, and 49° (Fig. 8). It was evident that 53° heated the eye too much but the others elicited retrolental temperatures, as well as rates of rise, that fairly well approximated temperature conditions in the microwave irradiated eyes (Fig. 9). A water bath temperature of 49° was therefore chosen for the final experiments.

Thirty-two experiments were performed in which the heated copper collar was applied directly to that part of the sclera overlying the region of the ciliary body and root of the iris. The surface temperature of the collar was found to be 0.5° to 1° less than the temperature of the water bath. In 14 cases, the water temperature was 49° or 50° C, applied for 40 minutes. In 5 more at 49°,

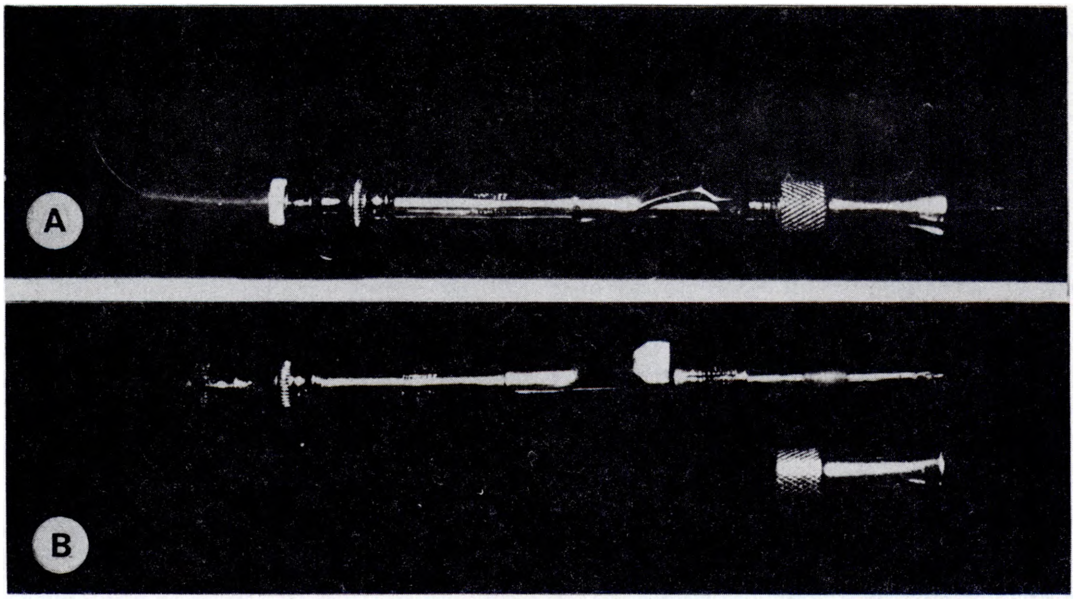

Figure 5. Adjustable propelled thermocouple. A. As used. B. Partly disassembled.

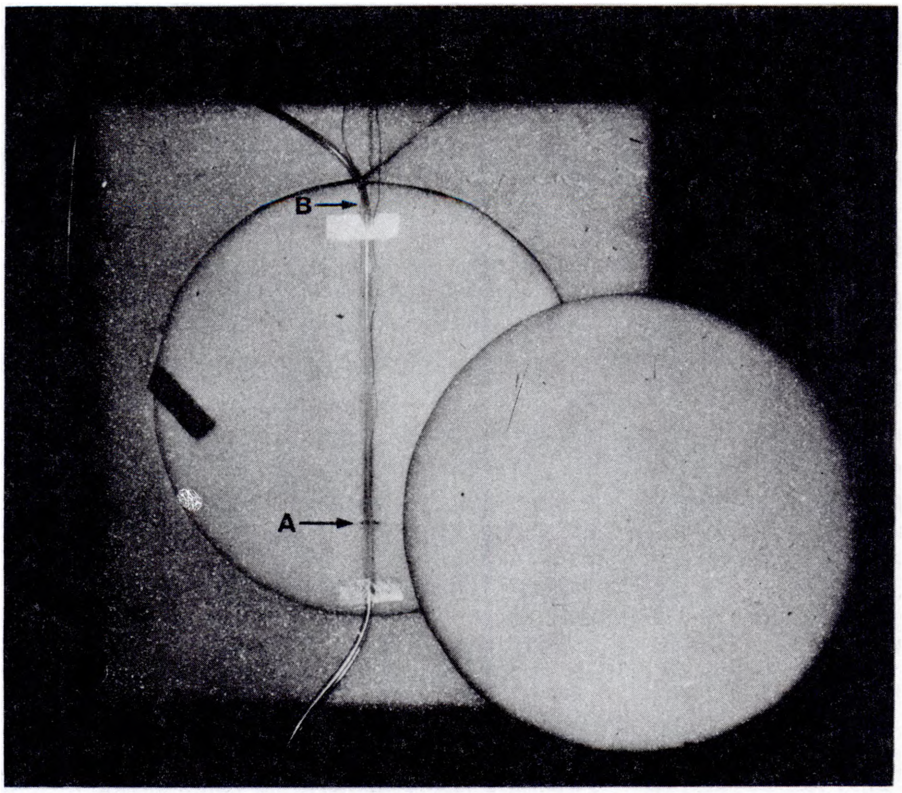

Figure 6. Eighteen inch diameter Styrofoam disc used for testing effect of EM field on thermocouple. Arrows point to thermocouple.

Table 1. Single irradiation of rabbit eyes

2.45 GHz, C.W. - 250 mW/cm^2

No. of exp'ts	Exposure duration	Posterior cortical banding	Granules along posterior suture	Posterior subcapsular opacity	Interval before any effect observed	Interval before opacity appeared	Observation period
4	20 min.	0	0	0	—	—	35 days
3	30 min.	2	0	0	24-48 hrs.	—	36 days
6	35 min.	5	2	0	24-72 hrs.	—	16 to 45 days
6	40 min.	6	4	5	24-48 hrs.	3 to 7 days	47 to 85 days

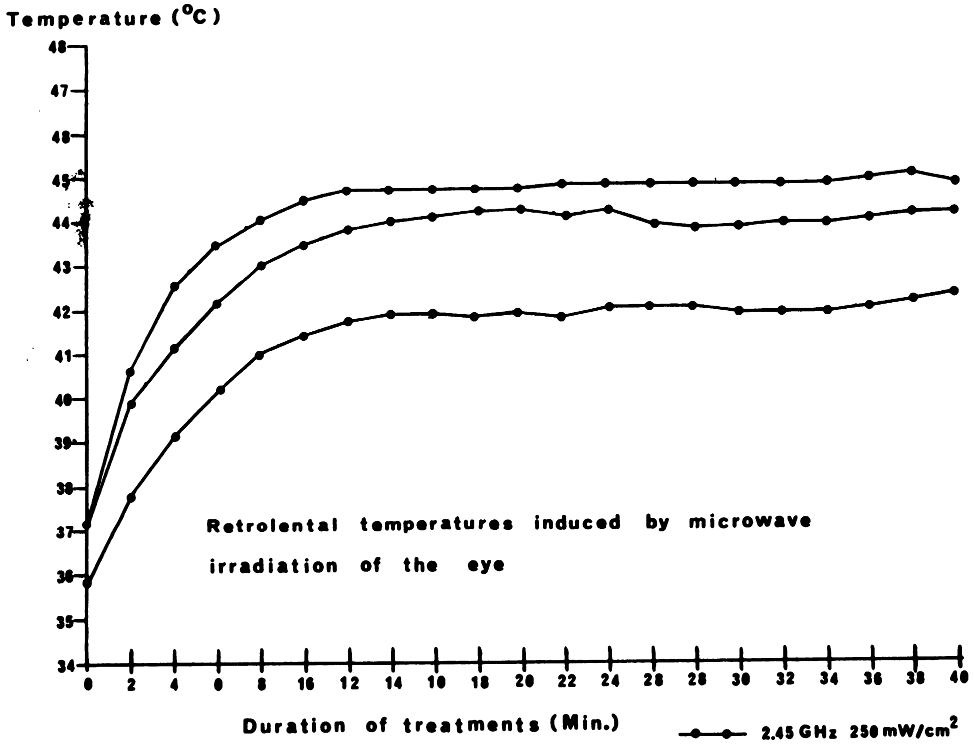

Figure 7. Retrolental temperatures induced in three rabbits by microwave irradiation of the eye. (2.45 GHz, C.W., 250 mW/cm^2 for 40 minutes)

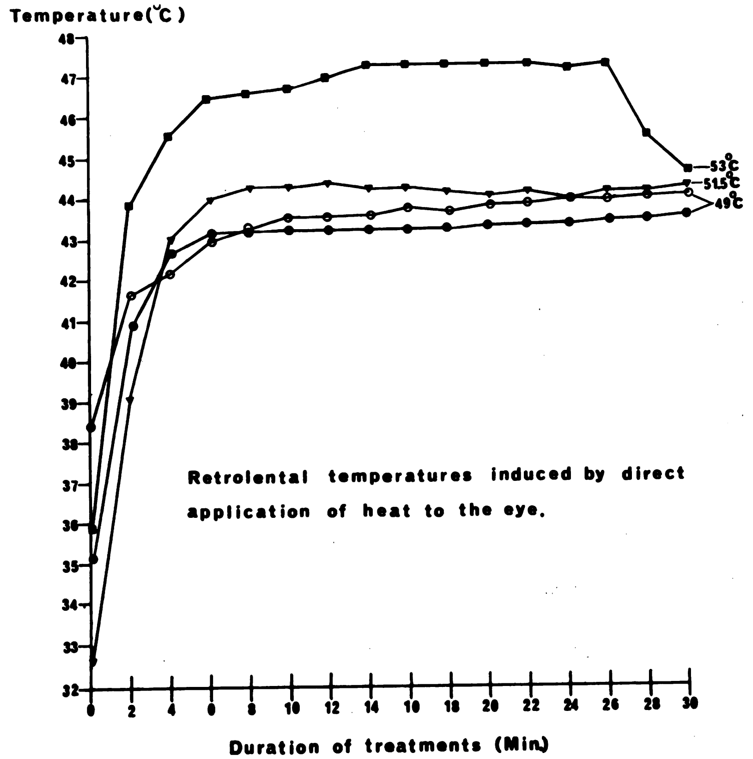

Figure 8. Retrolental temperatures induced in four rabbits by external application of heat directly to the eye for 30 minutes.

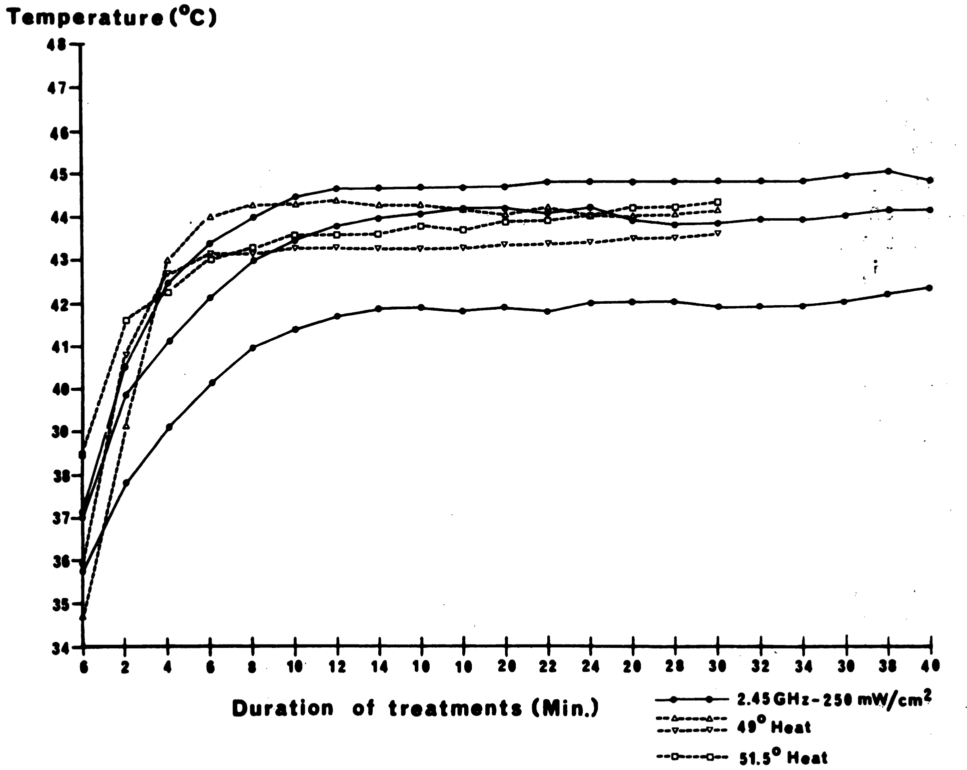

Figure 9. Retrolental temperatures induced in the eye by microwave irradiation (solid lines) and by direct application of heat (broken lines).

the eye was heated for 30 minutes. The remaining 13 experiments comprised 6 at 49° for 15 minutes and 7 at 52° for 15, 20, or 30 minutes.

The treatment was severely traumatic. Without exception, pupillary contraction occurred promptly even in eyes treated with a mydriatic just previously. The rate and degree of contraction varied. In one case, the pupil diameter changed from 8 mm to 2.5 mm in 3 minutes and in another, from 11 to 6 mm in 2 minutes. In all instances, maximal miosis was present after 40 minutes of heating. Other immediate responses included bulbar and palpebral chemosis, hyperemia of limbal and iris vessels, iridemia, and hemorrhages in rectus muscles near their insertions. Within 24 hours of being heated, almost all the eyes exhibited severe hyphema (hemorrhage into the anterior cavity), so that observation of the lens became impossible. In several animals, the eye suffered external infection. We therefore routinely applied medication immediately following the heating treatment and for several days thereafter. In some cases, the eye became necrotic and we sacrificed the animals.

Of 32 experiments, there survived 21 animals in which the lens could be observed at least 8 days or more after it had been subjected to heating (Table 2). In the development of microwave cataracts, the first signs of lens damage appear within 1 week in the posterior subcapsular cortex (21,24). In only 9 animals could the lens be viewed by ophthalmoscope and slit-lamp biomicroscope. The remaining 12 were sacrificed and the eyes enucleated and hemisected for immediate study under a dissecting microscope. In the 21 eyes examined, the posterior cortex of the lens remained clear in 18 of them. Two of the posterior opacities were in eyes heated by 52° water. In the 6 usable eyes heated for 40 minutes, there developed only one posterior opacity, which was first seen 6 weeks later. Opacities in the anterior cortex occurred in 5 of the 21 eyes.

Raising the Retrolental Temperature by Restricting Loss of Body Heat During Irradiation at Less than Cataractogenic Threshold

In irradiating the rabbit eye with 2450 MHz continuous wave radiation at an incident power density of 250 mW/cm^2, a single exposure of 40 minutes was found to be cataractogenic. Measurements made during such exposures showed increases in both retrolental and rectal temperatures (Fig. 10). Differences between them were similar in different animals. While the retrolental temperature rises some 7°, the microwave energy absorbed by the eye and by the nearby head region as well causes only about 2° elevation of rectal temperature. It should be noted, however, that this increase represents only the absorbed energy which the animal fails to dissipate.

The rabbit's principal means of heat transfer is by convection and radiation from its large ears. Wathen et al. (25) in a study of this mechanism, pointed out the following: "Loss of heat through convection and radiation, however, is possible only as long as animal surface temperature remains above environmental temperatures. When the situation is reversed, and environmental temperatures exceed the animal's surface temperature, additional surface area could become a liability to the animal, since heat would flow into, instead of out of, the animal at this time."

In view of this circumstance, it seemed reasonable to assume that by raising the environmental temperature of the rabbit's ears, we could thereby cause its body temperature to increase. This induced hyperthermia would necessarily raise the retrolental temperature, so that if we simultaneously irradiated an eye, we might produce in it a retrolental temperature characteristic of a cataractogenic microwave exposure, even though the microwave power applied during 40 minutes might be well below the 250 mW/cm^2 level required for cataract induction. We therefore measured retrolental and rectal temperatures in rabbits

Table 1. Effect on the rabbit lens of direct application of heat to the eye.

No of experiments	Water temp. (°C)	Heating period	Usable experiments	Observation period (days)	Opacities in lens			Post. cortex clear
					Anter. cortex	Equatorial	Post. cortex	
6	49°	15 min.	6	14-70	0	0	0	6
5	49°	30 min.	5	10	2	0	0	5
6	49°	40 min.	3	8-338	1	0	0	3
8	50°	40 min.	3	15-17	0	0	1	2
2	52°	15 min.	1	448	1	0	1	0
4	52°	20 min.	2	17-32	1	0	1	1
1	52°	30 min.	1	14	0	0	0	1
32			21		5	0	3	18

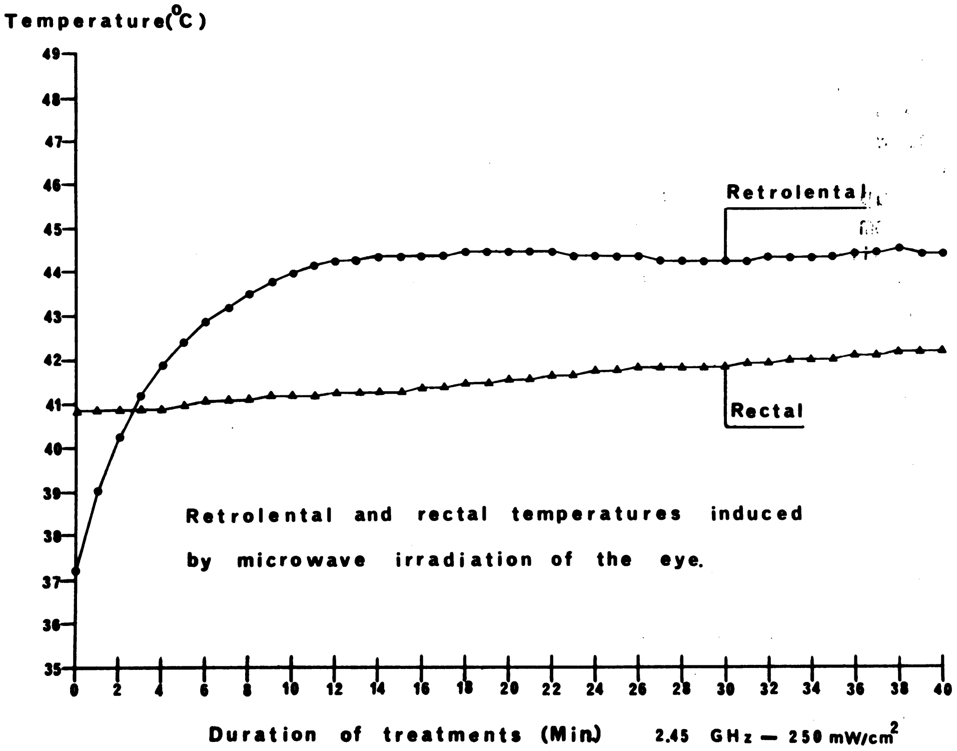

Figure 10. Retrolental and rectal temperatures induced by microwave irradiation of the eye. (Average of three rabbits)

during a 40 minute period when their ears were encased in an insulated polyethylene sack through which flowed air heated to 55° C. Averages and range of temperatures for 4 animals are shown in figure 11. Retrolental temperature increase averaged 3.2° and rectal, 2.4°. The course of the rectal temperature was strikingly similar to that occurring during a 40-minute microwave irradiation of the eye at 250 mW/cm^2 (Fig. 12). We therefore felt that if this rise in body temperature were to be augmented by an appropriate amount of microwave energy directed to the eye, we might be able to duplicate the retrolental temperature of a cataractogenic microwave exposure but at power densities well below the cataractogenic level. To that end, we needed to determine how much microwave power would be required to raise the retrolental temperature the desired amount over a 40-minute period and whether the added heat thus dumped into the body's circulatory system would evoke a parallel response in rectal temperature.

Accordingly, we performed two experiments in which the anesthetized rabbit was placed with the left eye 2 inches from the dipole of the Director "C," with the ears in the air sack and two themocouples in place, an intraocular one behind the lens and a rectal one. As air heated to 55° C flowed over the ears, the microwave power was constantly adjusted so that the retrolental temperature closely matched that of the 40-minute cataractogenic irradiation shown in figure 10, which was adopted as the "desired retrolental temperature." Rectal temperature was recorded every minute. Temperature recordings for the two experiments are shown in figure 13, with accompanying incident power densities.

The retrolental temperatures provided a satisfactory match and their relations to their respective rectal temperatures were similar. Since the latter were in close agreement, we adopted the average of the two as a "desired rectal temperature." Four more animals were similarly treated, except that microwave power was constantly adjusted to make the rectal temperature conform to the adopted "desired rectal temperature" and the retrolental temperature was recorded for comparison with the "desired retrolental temperature." Two of these cases are shown in figure 14. They were in good agreement and the method appeared to be a valid one to use.

Ten experiments were performed. Each rabbit, under sodium pentobarbital anesthesia, was placed in a Styrofoam holder in an anechoic chamber, with its left eye 5 cm from the Director "C" and its ears enclosed in the hot air sack. One thermocouple measured rectal temperature; air temperature in the sack was recorded from a second thermocouple. Air flow at 55° C was started and radiation switched on at an initial incident power density of 250 mW/cm^2. Rectal temperature was monitored each minute and microwave power adjusted to bring it to close agreement with the patten of the "desired rectal temperature" during the 40-minute exposure.

The procedure imposed severe heat stress on the animal so that when it was completed, prompt treatment was required for its survival. The animal's body was submerged in room temperature water (20° C) for 10 to 15 minutes, after which the fur was toweled and dried by moving air. Eyes were examined by ophthalmosocope and slit lamp over periods of 12 to 60 days. The results are summarized in Table 3, in which the column headed "Microwave dose (mW·min/cm^2)" represents the sum of the several power densities, each multiplied by the number of minutes it was in use. A dose of 250 mW/cm^2 in use for 40 minutes equals 10,000 mW·min/cm^2, which is 100 percent of the threshold cataractogenic dose.

Opacities in the posterior cortex of the lens developed in only three of 10 animals; in two of them, the microwave doses equalled 90 percent and 91 percent, respectively, of a cataractogenic dose. In the remaining case, a small posterior opacity was evident 13 days after only 76 percent of the cataractogenic dose.

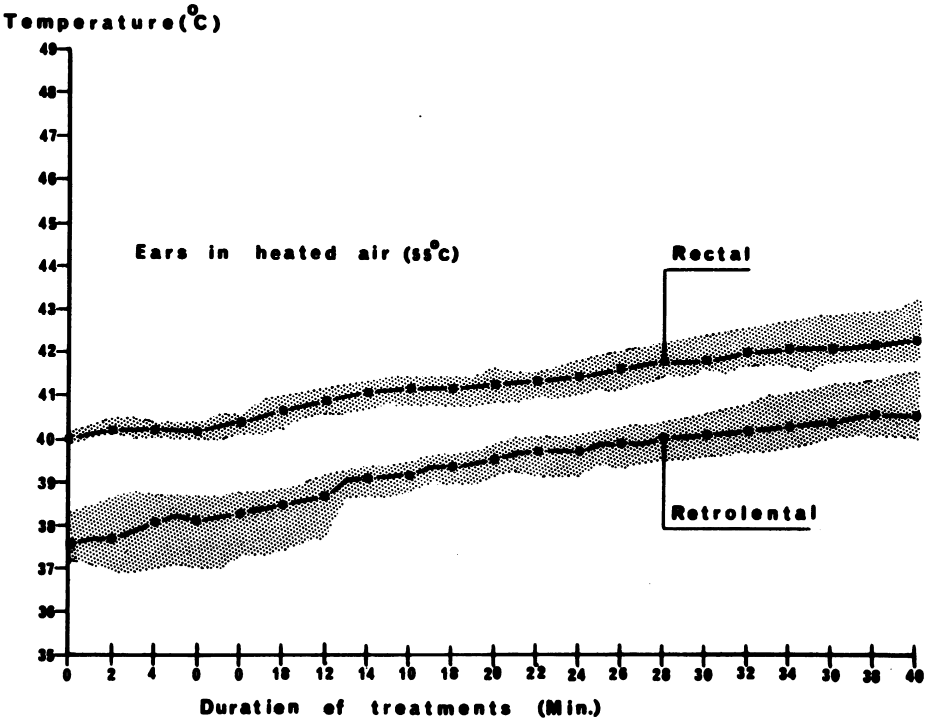

Figure 11. Retrolental and rectal temperatures when rabbit ears are subjected to 55° C air temperature. Lines represent average temperature of four animals; shaded areas disignate range of temperature.

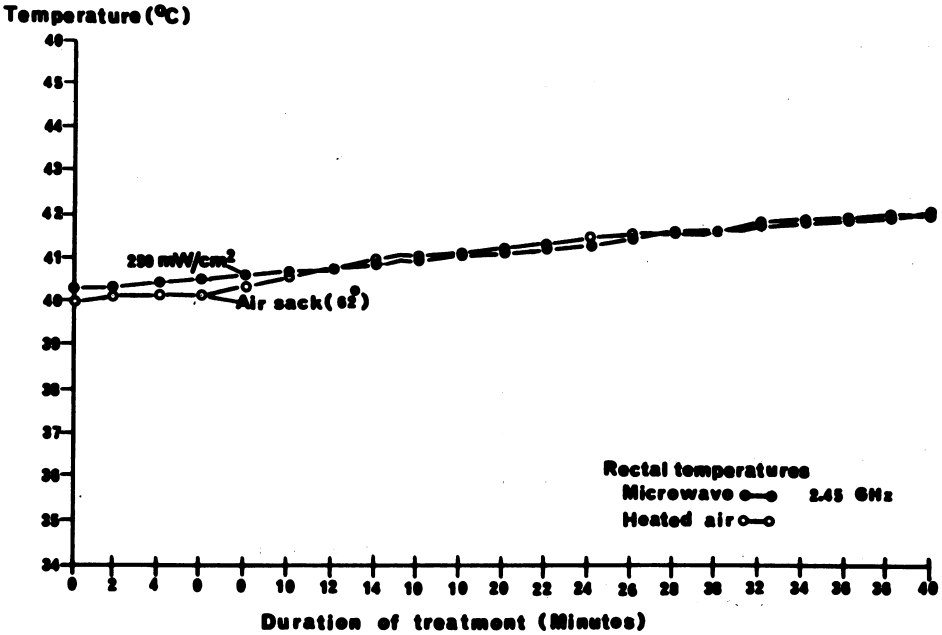

Figure 12. Comparison of rectal temperature induced in rabbits by microwave irradiation and by placement of the ears in heated air.

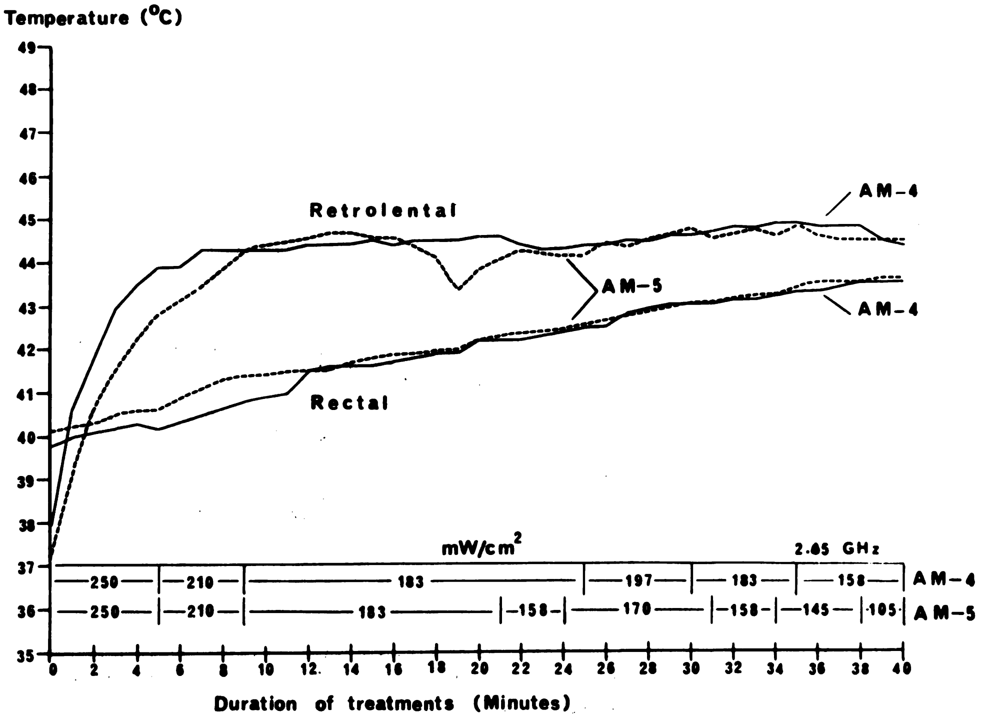

Figure 13. Retrolental and rectal temperatures of two rabbits (AM-4 and AM-5) with ears in 55° C air. The left eyes were irradiated at incident power densities adjusted to induce a retrolental temperature like that shown in figure 10 for a cataractogenic exposure.

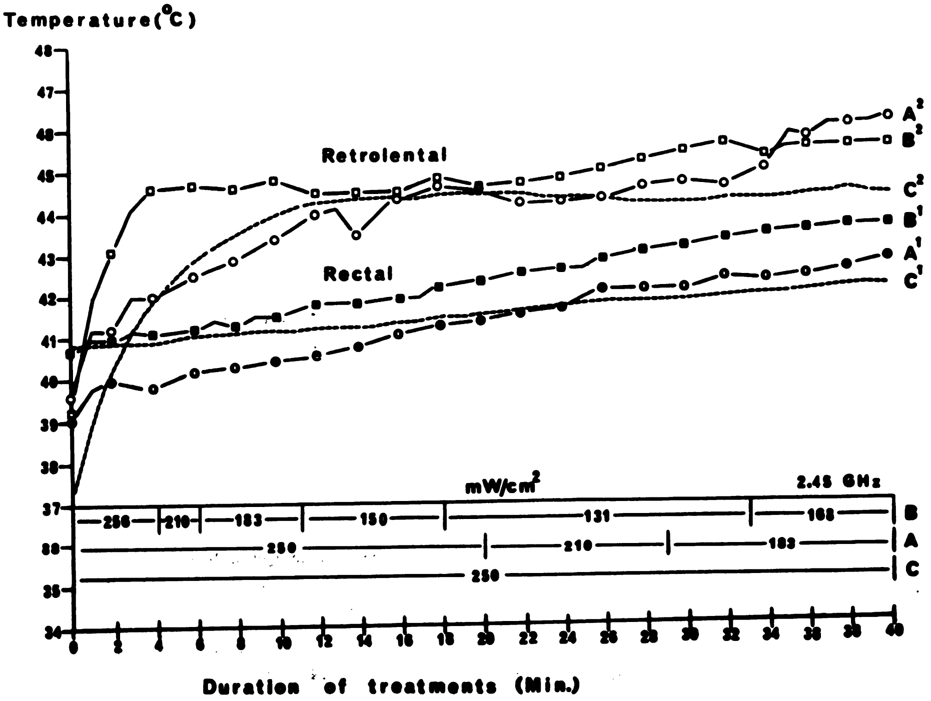

Figure 14. Retrolental and rectal temperatures of two rabbits (A and B) with ears in 55° C air. The left eyes were irradiated at incident power densities adjusted to induce a rectal temperature like that of the cataractogenic exposure shown in figure 10 and reproduced here as C^1.

It should be noted, however, that in 70 percent of the experiments, no opacities resulted.

Temperature Measurements

Because reliable information on temperature conditions was essential to the conduct of these experiments, the accuracy of our thermocouple measurements became a consideration of primary concern. To ascertain whether they were affected by the electric field or whether their presence in the field could cause localized hot spots, we undertook several tests.

1. If a thermocouple implanted in the eye during irradiation gives a false reading due to direct influence of the electric field, then when the microwave power is interrupted one should expect an abrupt change in the temperature reading. This does not occur.

2. A thermocouple probe was inserted in the eye through a puncture made superiorly 5 mm behind the limbus. Viewed through the cornea and lens, the junction was seen to be behind the posterior pole of the lens. Both thermocouple and lead were oriented vertically and hence were 90° to the Director "C" dipole. The eye was then irradiated and its retrolental temperature monitored continuously. After 40 minutes, irradiation was interrupted and a second temperature probe, the Adjustable Propelled Thermocouple (APT) was quickly inserted in the eye behind the lens. Within 20 seconds, the two probes registered temperatures alike within a half degree.

3. When two thermocouple probes, with orientation similar to that described above, were placed 34 cm apart in a length of 1/4-inch tygon tubing in Styrofoam, they registered identical temperatures when warm water was circulated through the tubing at a rate of 390 ml/min. With one probe (A) opposite the Director "C" dipole antenna and the other (B) completely outside the electric field, the antenna was energized to produce an incident power density of 250 mW/cm^2 at the position of probe A. As the circulating water was heated, temperatures registered by the probes continued to be identical.

Effect of Anesthetic

Objections have occasionally been voiced to the use of sodium pentobarbital for anesthesia when body or organ temperatures are experimental considerations. Michaelson et al. (26) have pointed out that in their irradiations of dogs anesthetized by pentobarbital sodium the rise in rectal temperature was greater (4.56° F) and more rapid than that occurring with anesthetics such as chlorpromazine or morphine sulfate and that the temperature decreased more slowly during post-irradiation cooling. Unmedicated dogs had a mean increase of 1.31° F during a comparable time period.

In the present experiments, we administered sodium pentobarbital (veterinary "Diabutal") intravenously. Its effect on the temperature of the normal non-irradiated rabbit was tested by monitoring rectal temperature continuously for 80 minutes, with anesthesia induced at 40 minutes (Table 4). In six experiments, during the first 40 minutes without anesthesia the rectal temperature in all animals varied within a degree, with the tendency toward a lowering temperature slightly greater than that toward a rising one. During the succeeding 40 minutes, under anesthesia, the variation was similar in extent but the direction of the slight trend was reversed. The effect of sodium pentobarbital on body temperature regulation in the normal rabbit therefore seems to be minor. Were it to be a factor in raising the core temperature during microwave irradiation, however, it would nicely abet the purpose of our experiments.

Table 3. Effect of high body temperature during microwave irradiation of the rabbit eye at below cataractogenic threshold

Experiment number	Body temperature (°C rectal)				Microwave dose	Percent of cataractogenic dose	Posterior cataract induced in lens
	At end of: 0 min.	20 min.	40 min.	Total increase (40 min.)	(mW·min/cm^2)		
Cataractogenic exposure (40 min. at 250 mW/cm^2) (3 cases)	40.8	41.3	42.2	1.4	10,000	100%	Yes
	40.9	41.8	42.3	1.4	10,000	100%	Yes
	40.9	41.5	42.2	1.3	10,000	100%	Yes
AM-7	39.1	40.6	42.2	3.1	9,119	91%	Yes
AM-8	39.4	41.5	43.1	3.7	8,675	87%	No
AM-10	39.3	42.0	44.0	4.7	7,507	75%	No
AM-11	39.1	41.8	44.0	4.9	8.012	80%	No
AM-12	39.3	41.7	43.4	4.1	8,325	83%	No
AM-13	39.3	41.6	43.3	4.0	8,660	87%	No
AM-14	40.0	42.3	43.6	3.6	8,098	81%	No
AM-15	40.0	41.8	43.5	3.5	9,040	90%	Yes
AM-16	39.8	42.0	43.8	4.0	7,588	76%	Yes
AM-17	39.9	41.9	43.5	3.6	8,702	87%	No

Table 4. The effect of sodium pentobarbital anesthesia on rectal temperature in the rabbit

	Experiment No.	R-1	R-2	R-3	R-4	R-5	R-6
	Body weight (kg)	2.20	2.15	2.20	2.60	2.10	2.00
	Rectal temp. (C°) after:						
Resting animal; no medication	0 min.	39.9	40.0	40.1	40.5	39.9	40.4
	10 min.	39.4	39.8	40.6	40.7	40.1	40.3
	20 min.	39.3	39.8	40.3	40.4	40.1	40.6
	30 min.	39.2	39.7	40.3	40.4	40.2	40.4
	40 min.	39.2	39.5	40.4	39.9	40.1	40.7
Sodium pentobarbital anesthesia (35 mg/kg)	50 min.	39.5	40.1	40.3	40.3	40.4	40.6
	60 min.	39.6	40.3	40.1	40.4	40.4	40.6
	70 min.	39.7	40.3	40.3	40.4	40.5	40.5
	80 min.	39.7	40.2	40.3	40.4	40.6	40.5
	Net change after 40 minutes	- 0.7°	- 0.5°	+ 0.3°	- 0.6°	+ 0.2°	+ 0.3°
	Net change after 80 minutes	- 0.2°	+ 0.2°	+ 0.2°	- 0.1°	+ 0.7°	+ 0.1°

DISCUSSION

As a result of the test described, we believe that our temperature measurements were generally accurate and that the thermocouples, as used, were not affected by the microwave field. We also believe that if they were responsible for any "hot spots" within the eye, these were so slight as to be unmeasurable and, if present, would be too insignificant, when compared to the total increase in retrolental temperature, to affect the outcome of the experiments. McAfee et al. (**27**) found that a stainless steel hypodermic needle containing a thermistor did not couple with the electric field of 9.31 GHz radiation when the probe was perpendicular to the electric field vector in air or in a solution having the same salinity as tissue. The thermocouple probes we used were similarly oriented and much smaller in diameter.

McRee (**28**), in suggesting a technique for determining energy absorption by using cooling curves measured after microwave irradiation of a specimen, found that the presence of a thermistor in the specimen during irradiation did not cause any change in absorption. On the other hand, Johnson (**29**) asserts that "conventional temperature-measuring instruments such as the thermometer, thermocouple or thermistor are all composed of metallic components which greatly distort the tissue fields and are thus inappropriate for making tissue temperature measurements during irradiation." Guy et al. (**18**) met the problem by installing in the eye a hollow glass probe through which a thermocouple could be passed immediately after interruption of irradiation. We achieved a similar end by using our adjustable propelled thermocouple, the penetrating needle part of which was thermally isolated so as to preclude loss of heat by conduction (**30**).

By computer modeling, Guy calculated the temperature rise and temperature distribution of an intact eye irradiated in the rabbit and found that at incident power levels of 100, 150, 200, and 300 mW/cm^2, the highest temperatures were localized on the sagittal axis of the lens near its posterior surface. At 200 mW/cm^2, the temperature was 42.4° C; at 300 mW/cm^2, it was 45.8° C. These results agreed with the measured changes in retrolental temperature. At 200 mW/cm^2, the average was just under 45° after 30 minutes of irradiation.

Extrapolation from these measurements yields an estimate of 43.4° C after 30 minutes at 250 mW/cm^2, which is close to our figure of 43.7°, the average of retrolental temperature in three rabbits irradiated for 40 minutes at 250 mW/cm^2.

Guy suggested a possible threshold cataractogenic temperature around 41° C in the eye and Kramar et al. (**19**) considered that a retrolental temperature above 41° C was required for production of lens opacities in the rabbit. Baillie (**7**) believed that when exposure to 2.45 GHz radiation produced an intraocular temperature in excess of 43° C, the resulting microwave cataract in the dog eye was a thermal effect.

In view of these various estimates for a threshold cataractogenic intraocular temperature, our figure of 43.7° C appears to be generous enough to allow some margin for error. If this tissue temperature is achieved through the application of an electromagnetic field and lens opacities result, the effect is a thermal one, according to a definition of terms by ANSI Subcommittee C95.4 (**29**). Conversely, if other means of providing equal heating will not produce lens opacities, then it cannot be termed a thermal effect.

In our first series of experiments, the eye was heated at the same rate, to the same extent, and for an equal period of time (Fig. 8) as it would experience during a cataractogenic microwave exposure (Fig. 10), the difference being that the equal heating was provided by other means; namely, direct application of heat to the surface of the eye. If microwave cataracts are solely the effect of heating, then all the heated eyes should have developed posterior cortical opacities. Of those eyes heated for 40 minutes, only one did, and this was after a latent period of 6 weeks, which is quite uncharacteristic for microwave cataracts. We suspect that the opacity in this case was due to the severe uveitis afflicting the eye.

According to the data of Guy et al. (**18**), the five eyes heated to 49° for 30 minutes also should have developed posterior cortical opacities. None developed during the 10-day post-irradiation period, whereas microwave induced ones are commonly well formed by then.

In our second series, we increased the body temperature by raising the environmental temperature of the ears and adding just sufficient microwave heating of one eye to bring its retrolental temperature up to the level which accompanies a cataractogenic exposure (Fig. 13). Although the microwave dose varied from 75 to 91 percent of a cataractogenic one (Table 3), it is a reasonable assumption that the retrolental temperatures in all 10 experiments approximated the desired level. In only three of 10 animals did opacities develop in the posterior cortex of the lens; in two of them, the microwave doses were 90 percent and 91 percent of the 40-minute cataractogenic dose. In the third case, with only 76 percent of the cataractogenic dose, opacities could be seen in the posterior subcapsular cortex of the lens 4 days after heating and irradiation.

The results of both series of experiments provide no support for the viewpoint expressed in the statement: "Insofar as the biological effects of microwave radiation are concerned, the only effects that have been produced can be reproduced by the application of thermal energy to the animal or to man." (**31**) On the contrary, they offer firm support to the conclusion that a lens opacity which develops after exposure of the eye to microwave radiation definitely is not simply a thermal effect.

Reviewing the present state of our knowledge on experimental microwave cataractogenesis, we find the following:

1. Our experiments, together with those of Kramar et al. (**22**), demonstrate that increased intraocular heat is not alone the factor responsible for the production of lens opacities.

2. The experiments reported by Baillie (**7**) and Kramar et al. (**19**) show that microwave radiation, without increased intraocular heat, cannot alone induce cataract formation. One might wish that Baillie's source of microwave power could have been regulated, for with only a 20-second exposure difference between no effect at all (55 sec.) and immediate coagulation of the lens (75 sec.), he had very little room in which to maneuver. Nonetheless, it remains a fact that in each of the four experiments he performed on dogs whereby both eyes were similarly irradiated, the eye exposed to microwaves while the animal was kept in a state of hypothermia was unaffected but the other eye, irradiated while the body temperature was normal, developed lens opacities. Kramar et al. (**19**) subjected the right eyes of rabbits to known cataractogenic levels of 2.45 GHz radiation with the animals under conditions of controlled general hypothermia. In the nine experiments, retrolental temperature was below 41° C and in no case did the acute exposure cause development of a lens opacity.

In both Baillie's and Kramar's experiments, cataractogenic levels of power, applied for a duration of time adequate to cause induction of lens opacities, failed to evoke this effect in the absence of elevated intraocular temperature.

Summarizing the situation briefly, it appears that neither heat without microwaves nor microwaves without heat can cause lens opacities. It has also been amply demonstrated that microwaves and heat together can be effective only when they act over a certain time period. We therefore suggest that microwave cataracts represent the effect of an adequate level of microwave power acting within a limited range of elevated intraocular temperature during a certain duration of time. These three factors can vary with respect to each other but ony within certain limits. What those limits are and the extent to which each factor can be varied without loss of cataractogenic effectiveness remains to be determined.

CONCLUSIONS

Lens opacities resulting from acute exposure of the eye to microwave radiation are not simply the manifestation of a thermal effect.

We suggest that three factors are involved and that they are an adequate microwave power acting within a limited range of increased intraocular temperature for a certain duration of exposure.

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OPEN DISCUSSION

DR. ALBERT: How were these sections cut? You said that they were 1/14000 of a millimeter, and they look more like 10 or 15 microns.

DR. CARPENTER: No, I said that they are 14 microns, and a micron is a one thousandth of a millimeter.

DR. ALBERT: Regarding your opening remark concerning a question on Dr. Hirsch's paper, how soon after the radiation did you look at those retinas?

DR. CARPENTER: That was some time after.

DR. ALBERT: Days, hours?

DR. CARPENTER: I have never made any study of consecutive changes following radiation.

DR. ALBERT: When you say that the retina looked normal, what time after radiation did you look at it to call the retina normal?

DR. CARPENTER: A month, two months. However, I have seen a fibrosed retina in eyes that had been heavily irradiated.

DR. ATHEY: Have you done any histology on lenses that obviously did not have cataracts to see if any of these same processes were taking place, for instance, in just barely subthreshold categories.

DR. CARPENTER: Yes, In this whole study, half of the lenses had been irradiated and the other half had not been. I studied over 100 lenses; half of them had been irradiated and were the controls. I have studied normal eyes, too.

DR. ATHEY: I am sorry, I meant exposed eyes that did not develop cataracts. Did you look to see if any of these processes leading to cataracts took place but did not finalize.

DR. CARPENTER: No.

DR. JUSTESEN: Dr. Carpenter, I did not hear an interpretation. I wonder if you would like to favor us with one.

DR. CARPENTER: I interpret it this way: when you have the same temperature as is attained in a cataractogenic exposure that produces a cataract, and a cataract does not develop, then a microwave cataract is not simply a result of heat. If it were caused merely be heat, a cataract would have resulted in every one of those cases, and it did not.

DR. JUSTESEN: That would not exclude the possibility of peripheral heating being involved in the formation of the cataract, would it?

DR. CARPENTER: Peripheral from where?

DR. JUSTESEN: I assume that an organism, other than the experimental circumstance that might theoretically be developing the microwave cataract, is not having its eye exclusively irradiated, rather the whole body is; is it not?

DR. CARPENTER: No, it was the head.

DR. JUSTENSEN: In other words, all of Milt Zaret's alledged 12 annotated bibliographies are people who had exclusive radiation of their eyeballs?

DR. CARPENTER: He did not know. They had a history of perhaps having owned a microwave oven. However, your point is that if you heat the whole body you might get a cataract. In that case, you had better not take a hot bath.

DR. MICHAELSON: I think the results of this experiment are going to be very difficult to interpret, and I will point out some things that I think many people do not realize. You are using anesthetized rabbits, am I correct?

DR. CARPENTER: Yes, and you are going to say that anesthesia affects the temperature control.

DR. MICHAELSON: That is one point.

DR. CARPENTER: And that is what we wanted; we wanted to be able to raise the animal's eye temperature.

DR. MICHAELSON: What you actually are showing, then, is a rising eye temperature in the presence of a falling core temperature. In other words, if you had left the animal alone on that table, his temperature would have been dropping progressively during the entire period.

DR. CARPENTER: No.

DR. MICHAELSON: Most certainly. What kind of anesthesia?

DR. CARPENTER: Nembutal.

DR. MICHAELSON: Yes, definitely. There is no question about it.

DR. CARPENTER: How much temperature drop do you measure?

DR. MICHAELSON: We have measured a drop of as much as 2° or 3°, sometimes 4° in a rabbit; and in a dog we have measured as much as 5°; this is well known.

DR. CARPENTER: I do not see how this is significant in the present instance.

DR. MICHAELSON: There is another point though; this is just part of the problem. You have a tremendous shift in gradient going on in the animal; it has been shown that when you cause this type of shift in gradient, you get what is known as paradoxical cooling--known for extremities, for example. But as the body temperature is going up, the temperature in the extremities is going down--producing tremendous shunting of blood or distribution of the heat. I do not know what happens in the eye at this time; however, the important point is that as the temperature is dropping as a result of the anesthesia, a tremendous burden is imposed on this animal and that is why you are getting this very nice increase. I think people must realize that there is a tremendous shift in the thermal regulation of these animals. Even if you take these temperature measurements and you get the same temperature measurements at a certain part of the body between the animals heated with this air, microwaves, or whatever it is; it does not mean that the temperature at that posterior subcapsule is going to be the same.

DR. CARPENTER: Why doesn't the same shift go on in the animals that are treated exactly the same way and in which we measured the retrolental temperature.

DR. MICHAELSON: No, you cannot do it; that is what I am saying. There is paradoxical cooling in many parts of the body.

DR. CARPENTER: Yes, well we don't worry about the arms and the legs getting cataracts. Why do you differentiate between one group of animals and another group of animals, both treated the same way?

DR. MICHAELSON: But they are not the same.

DR. CARPENTER: What is the difference?

DR. MICHAELSON: In one case you are introducing microwaves right into the head or through the eye; I do not know what you are doing to the hypothalamus at that point.

DR. CARPENTER: All of the animals received microwaves.

DR. MICHAELSON: Alright, you have a point there. The whole question of paradoxical cooling is extremely important. It has been shown, for example, if you put a thermal load around the hypothalamus (I think it is in sheep),there is a drop in body temperature. The important thing I am trying to point out is that we have to be very careful how we interpret these experiments because of problems encountered in the anesthetized animal if thermalization is introduced in the presence of dropping body temperature; there are tremendous shifts going on.

DR. CARPENTER: Do you get a drop in body temperature and a rise in rectal temperature?

DR. MICHAELSON: No, I am talking about core temperature measured at the colon.

DR. CARPENTER: That is where we measured it. As I showed you on the chart, the temperature goes up.

DR. MICHAELSON: Exactly, that is the point that I am trying to make; therefore, you are getting tremendous shifts in the animal.

DR. CARPENTER: The shift we wanted was an increase in core temperature to affect the eyes, to which we added some more heat.

DR. MICHAELSON: Your core temperature increase is a reflection of integration of all the temperatures in the body, except near the surface where you have a gradient; therefore, it is very difficult to make interpretations in the presence of all these factors.

DR. CARPENTER: It is difficult even though you measure the core temperature?

DR. MICHAELSON: Most certainly.

DR. CARPENTER: Are you saying, then, that the problems are insoluble?

DR. MICHAELSON: No, I am not saying that. Let's be careful how we interpret these things. I am telling the people in the audience to be very careful how they interpret this kind of data because it is very complicated; that is all I am saying.

DR. PHILLIPS: I think I see your point, Dr. Carpenter; I think other people are missing it. I think what you demonstrated very nicely was separating a pure thermal response in terms of cataracts.

DR. CARPENTER: That was the aim.

DR. OSEPCHUK: Could you clarify for me and the audience what you matched with the microwave case as compared with the other case; was this the temperature time profile as measured where?

DR. CARPENTER: Rectally.

DR. OSEPCHUK: Rectally; not in the lens?

DR. CARPENTER: We first measured a number of animals rectally and in the vitreous and saw the difference between them.

DR. OSEPCHUK: Yes, but the rectal temperature is not necessarily uniquely related to lens temperature; it depends upon the way the heating is applied.

DR. CARPENTER: Under the conditions of the experiment, it was so related; I showed you a curve to indicate that.

DR. OSEPCHUK: Did you make measurements of temperature/time profile in the lens for both cases and compare them? Was it experimentally determined by directly measuring the lens temperature that the lens temperature time profiles were the same?

DR. CARPENTER: Yes, however, you cannot go in and touch the lens; if you do you will get a cataract whether or not there is heat. If you scratch the capsule of the lens you will get a cataract.

DR. OSEPCHUK: What I am saying is that you are depending upon some indirect references in terms of referring rectal temperature to eye temperature, and so forth.

DR. CARPENTER: It is the sort of "indirect" as when the doctor takes your temperature and you have a temperature of 103°, and he says, "you've got something going on in there we have to take care of." If you take the temperature at all points of your body, it might not be 103°; but you don't want him to send you home, do you?

DR. OSEPHUK: The other point is that the temperature profile in space, of course, is also quite different in the two cases, correct? The thermal gradients in two cases in the eye are different.

DR. CARPENTER: Different under what conditions?

DR. OSEPHUK: During the heating period.

DR. CARPENTER: The temperature rises?

DR. OSEPHUK: No, I am talking about the gradient, the derivative of temperature with distance in various directions.

DR. CARPENTER: Gradient across where?

DR. OSEPHUK: Anywhere, across the lens particularly, but in all of those areas where something might happen.

DR. CARPENTER: Do you mean across the capsule, across the epithelium, or across the whole lens?

DR. OSEPCHUK: Across any or all of those. However, my point is that they are going to be different in principle because one has penetrating microwave absorption, and for the same reason that a hamburger cooks differently in a microwave oven than on a hotplate.

DR. CARPENTER: They all have microwave absorption.

DR. OSEPCHUK: Yes, but they have different degrees of absorption. In other words, the whole point is whether there was less microwave in the second case because of the adjunct method of heating, therefore, there are different patterns of heating.

MR. SCHNEIDER: Dr. Carpenter, I believe it was on your last slide where the four animals that were exposed to the 10,000 milliwatts per centimeter squared minutes got cataracts and most of the other animals did not. Were those four animals in the same kind of thermal enclosure with bags over their ears as the figures show?

DR. CARPENTER: Do you mean with no hot air?

MR. SCHNEIDER: Or with hot air.

DR. CARPENTER: No, because that would not be comparable.

MR. SCHNEIDER: That might be a way to resolve this business, essentially.

DR. CARPENTER: When we do that, then we will just go to the next experiment that I reported--that is, the whole group is given less microwave. If you give the rabbits the hot air on the ears and 250 milliwatts of microwave for 40 minutes you would give them heat stroke. As soon as the exposure was over, we had to immerse them in water to get the rectal temperature down to 41° so they would survive. I do not see how your experiment could be performed.

MR. SCHNEIDER: I am just suggesting that under those circumstances the thermal differences between the two groups would be minimal because there is not that much difference in the microwave power.

DR. CARPENTER: Yes there is.

DR. SCHNEIDER: Twenty percent.

DR. CARPENTER: Yes, but it's the effect on the eye.

DR. MICHAELSON: The temperature measurements in the eye are made at the end of exposure; they are not made during exposure.

DR. CARPENTER: The measurements are made continuously during 40 minutes of exposure.

DR. MICHAELSON: Oh, they are. I am sorry.

DR. CARPENTER: Otherwise we would not have the graph.

DR. MICHAELSON: No, I am talking about temperature measurements in the eye. You have no idea what kind of gradients you have been introducing into the vitreous.

DR. CARPENTER: Gradients across where?

DR. MICHAELSON: Temperature radiance across the volume of the vitreous; I think that is very important.

DR. CARPENTER: Do you mean from the lens to retina?

DR. MICHAELSON: Yes, certainly. I think that is extremely important; we will have to discuss this later.

FROM THE FLOOR: Yes, but we have been discussing this for 20 years, Dr. Michaelson.

HEAT-INVOKED CHANGES IN ASCORBIC ACID LEVELS ON THE RABBIT EYE*

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ABSTRACT

Levels of ascorbic acid have been determined in the microwave irradiated eye of the rabbit. Results from a current study do not agree with an investigation previously reported using the same exposure and analyses conditions. A possible explanation of the cause of these discrepancies is attempted. Heat alone is ruled out as the causitive factor involved in alterations of ascorbate in the microwave irradiation of the eye.

INTRODUCTION

Few investigators have been willing to address their research in the area of microwave cataractogenesis from a biochemical alteration approach. This lack of interest seems neglectful, especially, after it has been reported that a drop in ascorbic acid levels in the lens of rabbits was the first identifiable reaction to microwave radiation, and that this occurred before any visible signs of damage were present (1).

Let us briefly review the recent literature dealing with investigations of ascorbic acid in relation to microwave irradiation of the eye. Merola and Kinoshita (1) in 1960 reported that reductions in ascorbic acid of approximately 20 percent occurred in the lens of the rabbit sometime between 6-18 hours post irradiation. They did not continue their study beyond this 18 hour period nor did they investigate any times between the post irradiation in the acqeous humor and therefore ruled out the possibility that the reduction in the lens was due to a decrease in this fluid.

Keene (2) demonstrated decreases in ascorbic acid in irradiated rabbit lenses cultured in vitro. However, she states that the controls also lost ascorbic acid but at a slower rate than the irradiated lens. Her results were ambiguous with large differences being exhibited for the same culture periods.

Weiter et al (3) reported that cultured rabbit lens lost ascorbic acid when exposed to microwave radiation and that this loss was due to thermal increases with gradually increasing power densities. Since portions of the lenses during irradiation were exposed to the air one may question whether the decreases might have been due to surface evaporation and some cell death occurred, or the lens capsule became a "leaky" membrane which resulted in losses.

Other investigators (4) using labelled culture media have found no differences in the uptake of ascorbic acid between an exposed and control lens for periods up to 19 hours post irradiation.

Ferri (5) recently reported data on a study of ascorbic acid values in the vitreous, lens, and aqueous of rabbit eyes exposed to single and multiple microwave irradiations, and assayed at 5 minutes, 30 minutes, 12 hours, 18 hours, 1, 2, and 4 weeks post irradiation. Lens ascorbic acid levels appeared normal with

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the exception of about a 20 percent decrease at 1 week post irradiation for an accute exposure. This drop was primarily coincident with the appearance of lens opacities. Ascorbate levels in the vitreous humor remained within control limits for all post irradiation assay periods. Data accumulated on the aqueous humor indicated that levels of ascorbate were decreased in this fluid after an acute exposure and these levels continued to decrease from 5 minutes after the insult until a low was reached at 1 week post irradiation. Recovery to normal values occured by 2 weeks post irradiation and were still within control limits at four weeks after irradiation.

RESULTS AND DISCUSSION

The results obtained in this recent study on lens ascorbate were not in agreement with those reported by Merola and Kinoshita in 1960. An analysis of the data collected by Merola showed that there was a significant difference at the 99 percent confidence level between ascorbate levels in the right irradiated eye and the left control eye when analyzed at 18 hours post-irradiation. This difference was not apparent for this same post irradiation period in the current study. The irradiation facilities, equipment, exposure conditions, and analysis methodology used in both investigations were the same. However, in Merola's study the facility for irradiation and enucleation of the eye was located at some distance from the laboratory where the analyses of ascorbate were performed.

As a possible reason for the discrepancies we felt that the time elapsed between sacrifice of the animal, removal of the eye, and subsequent transportation to the laboratory for analysis of the specimen, could be of long duration compared to the entire operation of sacrifice, dissection, sample preparation, and analyses steps being performed in sequence and in order at one location. For our study, elapsed time from death of the animal to completion of analysis of all eye parts was 58 ± 8 minutes. One can assume that analysis time for both studies was equal, but that transportation would delay the assay schedule for the original study. To test the delay hypothesis, aliquots of a known standard ascorbic acid solution were taken at hour intervals over a period of 5 hours and titrated in the same manner as an actual sample. The titration method of Roe was used for analysis (6). The rate of decrease of dye used was 50 units/hour or the equivalent of 0.19 mg ascorbate/hour over the first 3 hours and 20 dye units/hour or 0.007 mg ascorbate/hour after 3 hours. This meant that after 1 hour approximately 20 percent of the ascorbic acid had become unavailable.

A statistical analysis of 76 duplicate assays of lenticular material from both irradiated and control eyes showed no significant differences with a time range between replicate analyses of 1-5 minutes. Table 1 shows that when the second assay of lens material was delayed one hour for both control and irradiated eyes, there was still no significant difference due to the delay. One must, therefore, assume that the elapsed time due to transportation of samples was not a causitive factor in obtaining differing results.

Table 1. Replicate assay of ascorbic acid in the rabbit lens

	Ascorbic acid - mg/100 g	
Description	t = 0 min.	t = 60 min.
Unexposed	13.5	12.2
	13.6	11.6
	11.2	10.3
	12.8	10.9
Exposed - 2.45 GHz, C.W. 5 min @ 500 mW/cm^2	17.0	19.2
	17.2	18.7
	18.6	17.9
	17.1	17.6

At 95% C.L. difference between t = 0 and t = 60 is not significant

Student t = 0.553 d.f. = 7

Gluathione, another reducing substance, is found in high concentrations in the lens of the eye. This substance shows early decreased levels in the lens in ionizing radiation produced cataracts. It also reacts with the dye used in the titration method for ascorbic acid; however, its rate of reaction is much slower than that for ascorbic acid. To test whether it was acting as an interfering in the titration, amounts of it equivalent to that normally present in the lens were added to a standard solution of ascorbate and the usual method of analysis performed. Differences in titer were not observed in solutions containing it from those where no glutathione was present. Therefore, it was concluded that glutathione did not interfere in this method of assay, and did not contribute any error to the accuracy of the titration. (See Table 2)

Table 2. Titration of ascorbic acid in the presence of gluthathione

Solution	Aliquot	Units dye used	
A	0.5 ml	77	
A	0.5 ml	76	
A	0.5 ml	80	
			78 average
A	0.5 ml		
B	0.4 ml	79	
A	0.5 ml		
B	0.4 ml	84	
A	0.5 ml		
B	0.4 ml	78	

80 average

A - .050 mg/ml ascorbic acid in 5% HPO$_3$
B - 1 mg/ml glutathione in H$_2$O

In the course of assaying for ascorbate in the eye parts, the lens was weighed immediately after enucleation and just prior to homogenization in a protein precipitant solution. This offered the opportunity of observing the relationship between the weight ratio of the irradiated and unirradiated lens and the severity of the damage to the lens produced by the radiation observed by slit lamp inspection just prior to sacrifice of the animal and removal of the eye for analysis. It was also possible to determine weight ratios at various times after exposure (such as at 5 minutes, 30 minutes, and 12 and 18 hours post irradiation) but before any visible damage would be apparent by slit lamp examination. This comparison was performed for those animals exposed to both a single acute dose and a repetitive subthreshold dose of microwaves.

Minimal, moderate, and maximum responses were as catagorized by Carpenter et al in previous studies (7). Minimal responses included small vesicules or granules along or near the post suture and possibly cortical banding - concentric bands of greater optical density separated by clear zones, and appearing in the posterior subcapsular cortex. Moderate responses showed small circumscribed opacities, crescent shaped and well-defined, located in the posterior lens cortex. A maximal response was a diffuse opacity involving a large part or all of the posterior cortex.

Table 3 shows a comparison of the mean weight ratio of the controls to the mean ratios of the various degrees of observable damage. The nonobserved category refers to those animals whose lenses were removed a short time after exposure but before discernable injury was apparent. At the 95 percent confidence level there was no significant difference in the ratios for all categories for both single and multiple exposure conditions. At value of 1.76 with 18 degrees of freedom was obtained when the greatest differences were compared--that between the single maximal reaction and the controls. Therefore, it was concluded that no relationship existed between weight of the lens and severity of damage to it in this study.

Table 3. Comparison of lens weight and severity of damage in the rabbit eye

Lens response	Lens weight - X OD/OS ± 1 SD Single Exposure	 Repetitive Exposures
Controls-unexposed	0.9717 ± 0.1160	(13)
Non-observed	0.9770 ± 1447(43)	---------
Negative	1.0106 ± 0.315(3)	1.0277 ± 0.194(2)
Minimal	1.0036 ± 0.774(9)	1.0533 0764(3)
Moderate	0.9458 ± 0.0862(10)	1.0048 0.0318(20)
Maximal	0.8540 ± 0.1654(7)	1.0333 ± 0.0760(9)

( ) Number of animals

Merola and Kinoshita had raised the question in their study as to whether "an increase in temperature was the cause for the observed drop in ascorbic acid" in the lens. Ascorbic acid is quite unstable and is destroyed by heat in an alkaline solution. The pH of the aqueous humor is about 7.57-7.60. They checked the lens ascorbate at 1/2 hour and 6 hours after irradiation and found no drop in ascorbic acid and, therefore, ruled out heat as a cause of the reduction. They also tested the levels of ascorbate in the aqueous at 18 hours post irradiation and found no change had occurred, ruling out the possibility that ascorbate was unavailable to the lens and therefore, resulted in the lenticular decreases. In the present experiments the aqueous humor had risen an average of 10.1° C at the end of an acute exposure. As stated previously, reduced levels of ascorbate were found 5 minutes post irradiation and these levels continued to decrease until one week post irradiation. To ascertain whether heat alone was directly responsible for the drop observed in this fluid, the eyes of rabbits were heated without the use of microwaves and acid levels determined. The procedure was as follows:

(1) An aliquot of aqueous was removed from the left control eye of an anesthetized rabbit and analyzed for ascorbic acid.

(2) The ears of the rabbit were then immersed in a hot water bath at 50° C for 40 minutes thereby heating the aqueous of the eye via the circulatory system of the animal. Using an adjustable plunging thermocouple (8), the temperature of the viterous, taken at 1 minute after removal of the ears from the hot water was 39° C. The viterous was probably above 40° C while the ears were in the water bath, and therefore, it was assumed the aqueous termperature had also been elevated above normal at the same time.

(3) At the end of the heating period an aliquot of aqueous fluid was removed from the right eye and analyzed for ascorbic acid.

As an additional test of the action of heat on the aqueous, aliquots of this fluid were removed from both eyes of an anesthetized animal, one aliquot was heated to 55° C and both aliquots were analyzed for ascorbate. (See Table 4).

Table 4. Effect of heat on aqueous ascorbic acid levels in the rabbit eye

Description	Ascorbic acid - mg/100 ml		Ratio
	OD	OS	OD/OS
In vivo heating	19.4	18.3*	1.060
	40.6	33.5*	1.212
In vitro heating	19.4†	19.4	1.000
Unheated controls (12)			0.935 ± .0.95

* Aliquot removed prior to heating

† Heated to 55° C

These tests demonstrate that when the aqueous humor is heated without the use of microwaves as the energy source, the ascorbate levels are not degraded. This tends to negate the theory that heat alone causes reduced levels of ascorbate in the aqueous with resultant lens decreases. In fact, higher levels of ascorbic acidwere noted in the aqueous of eyes which had been heated via the circulatory system.

CONCLUSIONS

The preceding results strengthen and lend support to a theory that cataractogenesis is a secondary reaction and that a primary and initiating action for microwave damage may not be a direct effect on the lens of the eye or the fluids that bathe it but, rather, an absorption of energy at some other sensitive site which is later reflected as a damaged lens.

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OPEN DISCUSSION

DR. MICHAELSON: You asked the question about cataracts in rats; are you asking for microwave cataracts or cataracts in general?

MRS. FERRI: Either way.

DR. MICHAELSON: Do you know about the well-known galactose catatracts in rats?

MRS. FERRI: Yes, that is right, although I had not recalled that. But I was interested in whether anybody had ever attempted to induce microwave on cataracts in rats.

CONCLUDING REMARKS

Moris L. Shore, Ph.D.
Director
Division of Biological Effects
Bureau of Radiological Health, FDA

I would like to commend all of the workers who have joined us for this symposium on one of the programs that currently is being supported by the Bureau of Radiological Health. I think that we have made some very striking degrees of progress in many areas of investigation concerning the biological effects of microwaves. I hope that by the time we meet again to review these effects that we will have even more progress to show--perhaps in new areas including molecular biology.

I also would like to mention that there has been quite a bit of discussion in a number of areas about differences of opinion and views with respect to interpretation of experiments and so forth. I do not believe that it is constructive to criticize the work of others purely on intuition; rather, we must develop a scientific base through experimentation. I want to thank you very much.

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